Improving Outcomes in Prostate Cancer

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Declaration

I declare that the work presented in this thesis is my own. I have stated the contributions of others within the following acknowledgement section

……..

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Abbreviations and Glossary of terms

Abbreviation	Description
ADT	Androgen deprivation therapy
Almac	Almac Diagnostics
Alt	Alternative
AR	Androgen receptor
AR-V	Androgen-receptor splice variants
ASCO	American Association of Clinical Oncology
AURKA	Aurora kinase-A, cell cycle regulated kinase involved in mitosis
BCDX2	Rad51B-Rad51C-Rad51D-XRCC2 Complex
BER	Base excision repair
BD	Twice a day
BRCA-m	BReast CAncer susceptibility gene mutated
BRCA-WT	BReast CAncer susceptibility gene wild type
BROCA	Gene panel used for screening for suspected hereditary cancer predisposition syndromes
CALGB	Cancer And Leukemia Group B, US based cancer research cooperative group
CAP	College of American Pathologists
CAPIRI	Capecitabine and irinotecan
Chr	Chromosome
CI	Confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CNA	Copy number alternation
Cox-2	Cyclooxygenase 2
CR	Complete response
CRC	Colorectal cancer
CRPC	Castration Resistant Prostate Cancer
CRUK	Cancer Research UK
CTC	Circulating Tumour cell
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumour DNA
CTU	Clinical Trials Unit
DFS	Disease free survival
DNA	Deoxyribonucleic acid
DSB	Double stranded breaks
EGFR	Epidermal growth factor receptor
EORTC	European Organisation for Research and Treatment of Cancer
EREG	Epiregulin
ESMO	European Society of Medical Oncology
F1	FoundationOne
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin embedded
FFS	Failure-free survival
FM	Foundation Medicine
FOLFIRI	Fluorouracil, folinic acid and irinotecan
gBRCA-m	Germline BRCA mutation
gBRCA-WT	Germline BRCA wild-type
GE	Genomics England
gHRD-m	Germline mutation in one or more HRD genes
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HR	Hazard Ratio
HRAS	HRas proto-oncogene, GTPase
HRD	Homologous recombination deficiency
IDH1	Isocitrate dehydrogenase 1

IFN-γ	Interferon-gamma
IHC	Immunohistochemistry
Il-10	Interleukin-10
IL-17	Interleukin-17
IL-4	Interleukin-4
IPP	Isopentenyl pyrophosphate
IQR	Interquartile range
ITT	Intention-to-treat
LHRH	Luteinising Hormone Releasing Hormone
LP	Likely pathogenic
M0	Non-metastatic
MAMS	Multi-arm multi-stage
mCRPC	Metastatic castrate-resistant prostate cancer
mCSPC	Metastatic castrate-sensitive prostate cancer
MHC	Major histocompatibility complex
MI	Myocardial infarction
MRC	Medical Research Council
MRN complex	MRE11–RAD50–NBS1 complex
MSI	Microsatellite instability
MSK-IMPACT	Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets
NCI	National Cancer Institute (USA)
NEPC	Neuroendocrine prostate cancer
NF-κB	Nuclear factor kappa B
NGS	Next-generation sequencing
NHEJ	Non-homologous end-joining
NHEJ	non-homologous end joining pathway
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIH	National Institute for Health
NSAID	Non-Steroidal Anti-inflammatory Drugs
NSCLC	Non-small cell lung cancer
NVALT	Nederlandse Vereniging van Artsen voor Longziekten en Tuberculose
OR	Odds Ratio
ORR	Overall response rate
OS	Overall Survival
P	Pathogenic
PARP	poly ADP ribose polymerase
PARPi	poly ADP ribose polymerase inhibitors
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PCSM	Prostate cancer specific mortality
PCWG	Prostate Cancer Working Group
PD-L1	Programme death-ligand 1
PFS	Progression-free survival
PGE2	Prostaglandin E2
PGE-M	Major urinary metabolite of PGE2 (PGE-M)
POR	Pooled Odds Ratio
PR	Partial response
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
pRR	pathological response rate
PSA	Prostate-Specific Antigen
PTGS	Prostaglandin endoperoxide synthetase
RANK	Receptor activator of nuclear factor kappa-B
RCT	Randomised Controlled Trial
REC	Regional ethics committee

RECIST	Response Evaluation Criteria In Solid Tumours
Ref	Reference
RP	Radical prostatectomy
RR	Relative risk
RS	Recurrence score
RT	Radiotherapy
RUO	Research Use Only
SMP2	Stratified Medicine Programme 2
SNV	Single Nucleotide variant
SOC	Standard of care
SRE	Skeletal related event
SSB	Single stranded breaks
SToPCaP	Systemic Treatment Options for Prostate Cancer; a collaborative meta-analysis group
STRATOSPHERE	STratification for RAtional Treatment-Oncomarker pairings of STAMPEDE Patients starting long –term Hormone treatment; a translational consortium conducting research in parallel with the STAMPEDE trial
SU2C-PCF	Stand-up to Cancer-Prostate Cancer Foundation; a funding initiative that has supported several large collaborative sequencing studies
SWOG	North American cancer cooperative group, formerly the Southwest Oncology Group
t170	Illumina TruSight Tumour 170 Panel
TCC	Transitional cell carcinoma
TCGA	The Cancer Genome Atlas
TGF-B	Transforming growth factor beta 1
TIL	Tumour infiltrating lymphocytes
TLR	Toll like receptor
TMG	Trial management group
TNF	Tumour necrosis factor
tNGS	Targeted next-generation sequencing
TP53BP1	Tumour-protein p53 binding protein 1
TSC	Trial steering committee
TTP	Time to progression
UCL	University College London
VEGF	Vascular endothelial growth factor
VUS	Variant of Unknown Significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
ZA	Zoledronic acid

Gene list

Abbreviation	Description
ABRA1	BRCA1 A complex subunit
AKT	Oncogene named after the viral oncogene v-AKT
APC	Adenomatous polyposis coli
ATM	Ataxia telangiectasia mutated gene
BARD1	BRCA1 associated RING domain 1
BRAF	v-Raf murine sarcoma viral oncogene homolog B
BRCA1	BReast CAncer susceptibility gene -1
BRIP1	BRCA1 interacting protein C-terminal helicase 1
CCND1	Cyclin D1
CDK	Cyclin dependent kinases
CDK12	Cyclin dependent kinase 12
CHEK2	Checkpoint kinase 2
CTNNB1	catenin beta-1
ERG	ETS-related gene
ETS	Erythroblast transformation-specific, a family of transcription factors
ETV	ETS translocation variants
FANCA	Fanconi anaemia, complementation group A
FLI1	Friend leukaemia integration 1 transcription factor, also known as transcription factor ERGB, is a member of the ETS transcription factor family
FOXA1	Forkhead box protein A1
JNK	c-Jun N-terminal kinases
KLK3	kallikrein-3, gene encodes PSA
MAPK	mitogen-activated protein kinase
MED12	Mediator complex subunit 12
MLH1	mutL homolog 1
MMR	Mismatch repair genes
MSH2	mutS homolog 2
MSH6	mutS homolog 6
mTOR	Mammalian target of rapamycin
NBN	Nibrin
NCOR1	Nuclear receptor co-repressor 1
PALB2	Partner and localizer of BRCA2
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
RAD51	RAD51 recombinase
RAD51B	RAD51 paralog B
RAD51C	RAD51 paralog C
RAD51D	RAD51 paralog D
RAD54L	RAD54L
RAS1	Ras family GTPase
RB1	Retinoblastoma 1, gene involved in cell cycle regulation
RNF43	ring finger protein 43
SPINK1	Serine protease inhibitor Kazal-type 1
SPOP	Speckle-Type POZ Protein
TMPRSS2	Transmembrane protease, serine 2, an androgen regulated gene
TP53	Tumour protein p53

List of clinical trials

Abbreviation	Description	Registration number
Add-Aspirin	Phase 3 placebo controlled trial od adjuvant aspirin in 4 cohorts: prostate, colorectal, breast and oesophageal	NCT02804815
AFFIRM	Phase 3 RCT of enzalutamide post chemotherapy in mCRPC	NCT00974311
ALSYMPCA	ALpharadin in SYMPtomatic Prostate Cancer, a phase 3 RCT of radium-223 in mCRPC	NCT00699751
APPROVe	Adenomatous Polyp Prevention on Vioxx Trial - rofecoxib chemoprevention RCT	NCT00282386
APRiCOT	Apricoxib in Combination Oncology Treatment - Lung	NCT00652340
ARASENS	Darolutamide in addition to ADT and docetaxel in metastatic castrate-sensitive prostate cancer	NCT02799602
ARCHES	A phase 3 study of enzalutamide vs. placebo plus ADT in metastatic castrate-sensitive prostate cancer	NCT02677896
CHAARTED	Chemohormonal Therapy in Metastatic Hormone-sensitive prostate cancer	NCT02677896
COU-AAA-301	Phase 3 RCT of abiraterone post chemotherapy in mCRPC	NCT00638690
COU-AAA-302	Phase 3 RCT of abiraterone pre chemotherapy in mCRPC	NCT00887198
CYCLUS	CY-cyclooxygenase-2 inhibitor, Chemotherapy, LUng cancer, Survival (Clinical Trial)	NCT00300729
ENGOT-OV16/NOVA	European Network for Gynaecological Oncological Trials/NOVA	NCT01847274
ENZAMET	Phase 3 trial of enzalutamide plus ADT versus ADT alone (+- docetaxel) in metastatic castrate sensitive prostate cancer	NCT02446405
FOCUS-4	A molecularly stratified trial programme in colorectal cancer	EUCTR2012-005111-12-GB
GECO	GEmcitabine-COxib in NSCLC	NCT00385606
GETUG-15	Phase 3 trial of ADT with or without docetaxel in metastatic castrate-sensitive prostate cancer	NCT00104715
LATITUDE	Phase 3 trial of abiraterone versus placebo in high-risk metastatic castrate-sensitive prostate cancer	NCT01715285
Matrix	National Lung Matrix Trial: Multi-drug Phase II Trial in Non-Small Cell Lung Cancer	NCT02664935
NCI-IMPACT	NCI-Molecular Profiling-Based Assignment of Cancer Therapy, a molecularly targeted basket trial	NCT01827384
NCI-MATCH	NCI-Molecular Analysis for Therapy Choice, molecularly targeted basket trial	NCT02465060
PATCH	Prostate Adenocarcinoma TransCutaneous Hormones	NCT00303784
PEACE-1	Phase 3 trial of ADT +/- docetaxel +/- local RT +/- abiraterone for metastatic castrate-sensitive prostate cancer	NCT01957436
PREVAIL	Phase 3 RCT of enzalutamide pre chemotherapy in mCRPC	NCT01212991
PROREPAIR-B	A prospective cohort study of DNA repair defects in metastatic castrate resistant prostate cancer	NCT03075735
RE-AKT	A study of Enzalutamide in combination with AZD5363 in patients with mCRPC	NCT02525068
RxRONDER	Rx for Positive Node, Endocrine-Responsive Breast Cancer	NCT01272037
STAMPEDE	Systemic Therapy in Advancing and Metastatic Prostate Cancer: Evaluation of Drug Efficacy	NCT00268476
SWOG 9346	Southwest Oncology Group trial 9346; phase 3 evaluation of intermittent versus continuous ADT in men with newly diagnosed castrate-sensitive metastatic prostate cancer	NCT00002651

TITAN	A phase 3 study of apalutamide plus ADT versus ADT in metastatic castrate-sensitive prostate cancer	NCT02489318
TOPARP	Trial of PARP Inhibition in Prostate Cancer, a clinical trial	NCT01682772
TRACERx	TRAcking Non-small cell lung cancer Evolution Through Therapy (Rx) (TRACERx)	NCT01888601
TRITON2	Trial of Rucaparib in ProsTate IndicatiONs; phase 2 non-randomised trial	NCT02952534
TRITON3	Trial of Rucaparib in ProsTate IndicatiONs; phase 3 randomised evaluation of rucaparib versus physician choice	NCT02975934
TROPIC	Phase 3 trial of mitoxantrone and prednisolone versus cabazitaxel and prednisolone	NCT00417079
UKGPCS	United Kingdom Genetic Prostate Cancer Study, a clinical trial	NCT01737242

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Hypothesis

Prostate cancer is a heterogeneous disease and men with metastatic castrate-sensitive prostate cancer (mCSPC) experience variable benefit from an increasing number of treatment options. Outcomes can be improved through moving away from a uniform approach and developing prognostic and predictive biomarkers to inform rational treatment selection.

Research aim

In this thesis I aim to develop a greater understanding of the results of the STAMPEDE clinical trial. I will explore how the data may inform treatment selection and consider how trials may adapt to evaluate molecularly-selected treatments.

Objectives

- To contextualise the celecoxib zoledronic acid (celecoxib-ZA) results in which the combination was synergistic in men with metastatic disease, despite no effect shown for either agent alone or in non-metastatic (M0) disease.

- To evaluate PSA response and PSA nadir as prognostic biomarkers able to risk-stratify patients when assessed after commencing ADT and completion of docetaxel respectively, to inform the use of additional therapies, such as docetaxel and abiraterone.

- To assess the feasibility of performing targeted next-generation sequencing (tNGS) using formalin-fixed paraffin embedded (FFPE) prostate tumour samples as a means of implementing molecular stratification.

- To explore the genetic profile of mCSPC and determine the prevalence of putative predictive biomarkers, specifically defects in genes involved in homologous recombination, to inform future trial designs of molecularly-selected therapies including poly ADP ribose polymerase inhibitors (PARPi).

Abstract

The treatment paradigm for metastatic castrate sensitive prostate cancer (mCSPC) is changing. The results of the STAMPEDE trial show intensified systemic therapy with docetaxel or abiraterone added to androgen deprivation therapy (ADT) improves overall survival (OS). However treatment benefit and tolerance vary, therefore prognostic and predictive biomarkers are required to improve patient outcomes.

Through performing a systematic review and meta-analysis I contextualised the intriguing celecoxib and zoledronic acid (celecoxib-ZA) results which showed a synergistic therapeutic effect that was only seen in metastatic disease. Secondly, I explored whether prostate-specific antigen (PSA) response, assessed after commencing ADT, and PSA nadir assessed after docetaxel completion, are prognostic of OS. Through collaboration with industry partners, I assessed the feasibility of performing targeted next-generation sequencing (tNGS) using formalin-fixed paraffin embedded (FFPE) prostate tumour samples. I explored the genomic profile of mCSPC to determine the prevalence of putative predictive biomarkers to inform the evaluation of therapies such as poly ADP ribose polymerase inhibitors (PARPi).

No other trials evaluating a cox-2 inhibitor with a bisphosphonate were identified. However, supported by pre-clinical data, an immunological mechanism mediated by γδ T cells is proposed to explain the observed synergy. This strengthens the need for future trials and informs parallel translational research. PSA response can be used to risk-stratify patients shortly after commencing ADT and may be used to inform the use of docetaxel. High PSA nadir, assessed after completion of docetaxel identified patients at high risk of death where additional systemic therapies e.g. abiraterone, should be evaluated. The genomic study found 94% (108/115) of samples had ≥1 pathogenic mutation although mutation frequencies remain low and pathway aberrations often co-exist, necessitating hierarchical allocation if used to guide treatment. The prevalence of HRD is clinically significant (15%) however the screening burden is considerable, compounded by variable sample quality, compromising the sequencing success rate (64%).

These data support the further evaluation of the combination of cox-2 inhibitors and bisphosphonates and the use of PSA-based outcomes in risk-stratification, whilst the genomic feasibility and prevalence data will inform future trial designs incorporating molecular stratification.

Impact Statement

The results of STAMPEDE show that celecoxib-ZA may significantly improve OS in men with mCSPC. However to influence clinical practice this surprising result would need to be supported by other evidence. Through undertaking a systematic review and meta-analysis I show that although there is currently a lack of supporting clinical data, pre-clinical data suggests an immunological hypothesis that may explain the observed synergistic therapeutic effect. Importantly, the proposed mechanism is not prostate cancer specific so may be relevant to other indications, for example metastatic breast or non-small cell lung cancer, potentially impacting future research priorities and patient outcomes across a range of cancers. I plan to disseminate these results through publication.

Two PSA-based biomarkers, PSA response and PSA nadir, assessed in patients receiving ADT alone and ADT and docetaxel respectively, are shown to be prognostic of OS. Both assessments are at clinically meaningful time points when they may inform the use of additional systemic therapy. To date, PSA response has been evaluated in small historical cohorts in mCSPC or castrate-resistant disease therefore this result represents a significant increase in our understanding. Following validation, this finding may impact on patient outcome through improved risk stratification, for example enabling the toxicities of docetaxel to be avoided in patients identified to be at less risk of progression, who could receive ADT alone. In a second analysis, PSA nadir >4ng/ml was associated with an increased risk of death despite docetaxel treatment. This finding adds to previous research that has shown this PSA threshold to be prognostic when assessed as an absolute value. The use of PSA nadir may impact future trial design through identifying a high-risk group in whom to evaluate additional systemic therapies, thus focusing research efforts on those patients with the greatest unmet clinical need. Additionally, these data may support future translational research, for example correlative analyses in the PSA response and PSA nadir cohorts may help identify predictors of relative ADT and docetaxel resistance respectively. I have presented these results to the STAMPEDE TMG and am preparing to disseminate further through presentation and publication.

Knowledge of the genomic profile of mCSPC is significantly advanced by the results of the sequencing analysis, which represents the largest cohort of mCSPC patients to be sequenced to date. The impact of this work will, in the first instance, be on the design of the next generation of trials, but ultimately may affect patient care through informing the implementation of biomarker-directed therapies. The prevalence and feasibility data

continue to inform how best to evaluate PARPi and other molecularly targeted therapies within the STAMPEDE trial and once presented, will be informative for others. Our experience of using different sequencing providers, technical protocols and data interpretation is also relevant to the future conduct of such trials. Through successfully leveraging industry support this project helped establish the required infrastructure for sample collection. The feasibility data obtained helped secure funding to support the STRATOSPHERE consortium, which continues to coordinate biomarker-focused research conducted in parallel to STAMPEDE, generating much needed data necessary to inform rational treatment selection in this setting.

Chapter 1 Introduction

1.1 Clinical overview

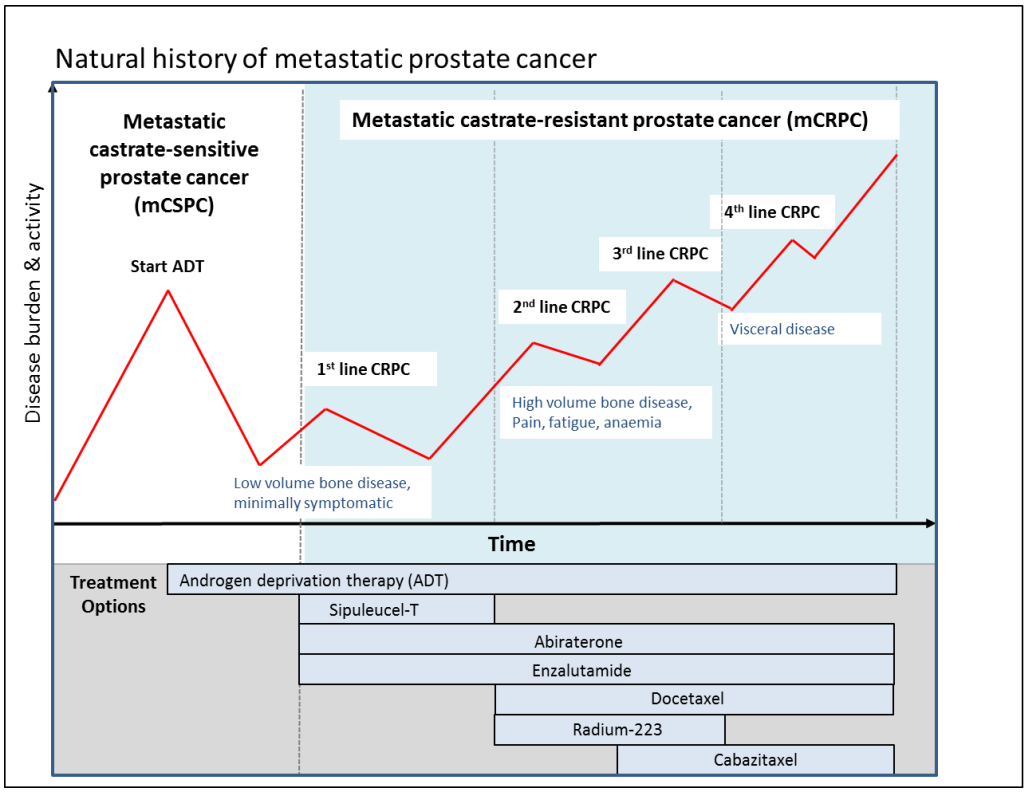
In the UK alone, each year over 45,000 men are diagnosed with prostate cancer (PCa) and there are over 11,000 prostate-cancer related deaths [1]. PCa has been described as "a heterogeneous disease that affects a heterogeneous population" [2]. One of the challenges in the management is appropriate risk-stratification, enabling informed treatment selection, balancing potential treatment benefit against the unavoidable costs and risks of toxicity. For the majority, PCa is a localised disease for which effective treatment strategies include radical treatment with radiotherapy (RT) or surgery, or active surveillance; all of which have been shown to offer excellent long-term outcomes[3]. However, around 10-15% of men will present with metastatic disease for which treatment is currently palliative and outcomes remain poor[4-6]. Numerous clinical trials are evaluating intensified therapeutic strategies for the management of metastatic prostate cancer, see **Table 1** and I will focus on this, the highest-risk group.

1.1.1 Systemic treatment of metastatic prostate cancer

PCa is hormonally driven as recognised by Huggins and Hodges who provided the first clinical evidence of the effectiveness of castration in the 1940s[7]. To this day, androgen deprivation therapy (ADT), typically achieved through the use of gonadotropin hormone releasing analogues, remains the cornerstone of the systemic management to which all subsequent therapies are added. Even if patients present with metastatic disease, over 90% will respond to ADT, however resistance is inevitable. Disease progression despite castrate levels of testosterone is termed castrate-resistant prostate cancer (CRPC) and is fatal in the vast majority; see Figure 1. for an overview of the natural history of metastatic prostate cancer. It is in the setting of mCRPC that the effectiveness of adding additional therapies to ADT was first demonstrated. There are now six treatments licenced for the management of mCRPC, all shown to improve overall survival (OS) when given with ADT; docetaxel, abiraterone, enzalutamide, cabazitaxel, radium-223 and Sipuleucel-T[8-15]. All may be given sequentially with ADT, yet despite the varying therapeutic mechanism of actions, all are used in unselected populations due to the lack of predictive biomarkers, see **Table 2**.

Figure 1: Overview of systemic therapies for metastatic prostate cancer

(adapted from Lorente *et al.*[16])



All six systemic therapies shown to be effective in mCRPC may be added sequentially to ADT which, until recently, was the only treatment for metastatic castrate-sensitive disease. Treatments licenced based on demonstrated survival gains include two taxane chemotherapy agents, docetaxel and cabazitaxel; two novel-AR targeted treatments abiraterone and enzalutamide, an alpha-emitting radio-pharmaceutical Radium-223 and Sipuleucel-T, an autologous dendritic vaccine.

Table 1: Ongoing phase III trials of systemic therapy given in addition to ADT in mCSPC

Target	Agent	Trial acronym	Registration	Patient group	Treatment details	Primary outcome measure(s)	Status
AR	Apalutamide	TITAN	NCT02489318	mCSPC (bone)	• ADT + Apalutamide • ADT+ Placebo	rPFS OS	Recruiting Accrual target: 1000 Est report date: Nov 2022
AR	Abiraterone	PEACE-1	NCT01957436	mCSPC	• ADT (+/- docetaxel) • ADT (+/- docetaxel) + abiraterone + pred • ADT (+/- docetaxel) +/- RT • ADT (+/- docetaxel) + RT + abiraterone + pred	OS (5.5yr) PFS	Recruiting Target accrual: 916 Est report date: Oct 2023
AR	Enzalutamide + Abiraterone	STAMPEDE	NCT00268476	mCSPC	• ADT • ADT + Abiraterone +Enzalutamide	OS	In follow up Accrued: 1976 Est report date: 2020
AR	Enzalutamide	ENZAMET	NCT02446405	mCSPC	• ADT (+/- docetaxel) • ADT (+/- docetaxel) +Enzalutamide	OS	In follow up Target accrual: 1100 Est report date: Dec 2020
AR	Daralutamide	ARASENS	NCT02799602	mCSPC	• ADT + docetaxel + Daralutamide • ADT + docetaxel + placebo	OS	Recruiting Target accrual: 1300 Est report date: Aug 2022
AR	Enzalutamide	ARCHES	NCT02677896	mCSPC	• ADT (+/- docetaxel) + Enzalutamide • ADT (+/- docetaxel) + placebo	rPFS	Recruiting Target accrual: 1100 Est report date: Dec 2023
Non-AR	Metformin	STAMPEDE	NCT00268476	mCSPC	• ADT (+/- docetaxel) • ADT (+/-docetaxel) + metformin	OS	Recruiting Target accrual: 1800 Est report date: 2025

Key: ADT, Androgen deprivation therapy; AR, Androgen receptor; Est, estimated; OS, overall survival; rPFS, radiological progression free survival; RT, radiotherapy; Pred, Prednisolone; PFS, progression free survival.

Table 2: Treatments shown to improve survival in mCRPC

	Treatment	Trial	Year	Comparator	Median survival benefit (months)	Hazard ratio
	Docetaxel	TAX 327[8]	2004	Mitoxantrone	2.9 (19.2 vs. 16.3)	HR 0.76 (0.62-0.94)
	Sipuleucel-T	IMPACT[15]	2010	Placebo	4.1 (25.8 vs. 21.7)	HR 0.78 (0.61-0.98)
Post-docetaxel	Cabazitaxel	TROPIC[13]	2010	Mitoxantrone	2.4 (15.1 vs. 12.7)	HR 0.70 (0.59-0.83)
	Abiraterone	COU-AAA-301[10]	2011	Placebo	4.6 (15.8 vs. 11.2)	HR 0.81 (0.70-0.93)
	Enzalutamide	AFFIRM[11]	2012	Placebo	4.8 (18.4 vs. 13.6)	HR 0.63 (0.53-0.75)
	Radium-223	ALSYMPCA[14]	2013	Placebo	3.6 (14.9 vs. 11.3)	HR 0.70 (0.55-0.88)
Pre-docetaxel	Abiraterone	COU-AAA-302[9,17]	2013	Placebo	4.4 (34.7 vs. 30.3)	HR 0.65 (0.54-0.77)
	Enzalutamide	PREVAIL[12,18]	2014	Placebo	2.2 (32.4 vs. 30.2)	HR 0.77 (0.67-0.88)

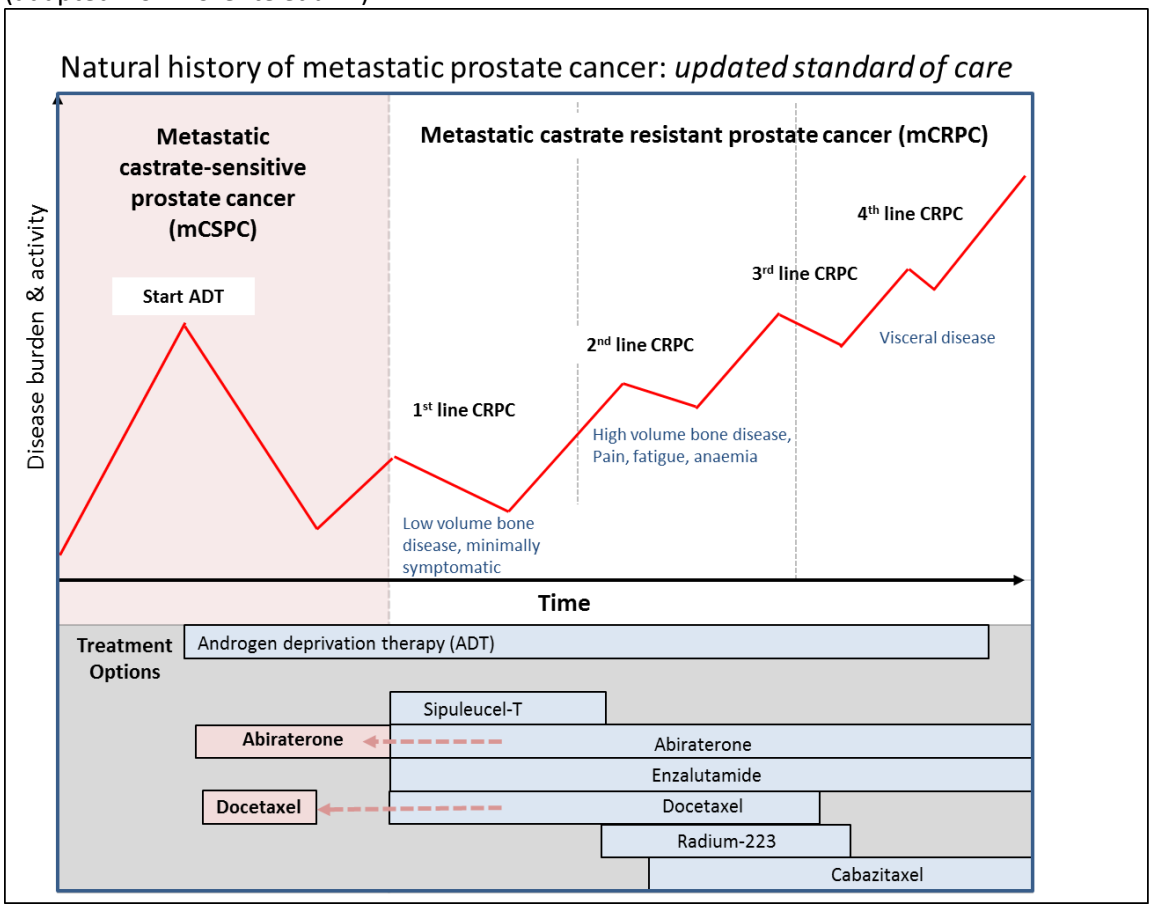
1.1.2 Changes in up-front systemic therapy

Between 2015 and 2016 the results of three trials provided level 1 evidence that the greatest absolute benefit of docetaxel and abiraterone, both known to be effective in mCRPC, may be gained when given in the castrate-sensitive setting, see **Figure 2**[19-21]**.** The addition of docetaxel to ADT was shown to improve survival in metastatic castrate-sensitive prostate cancer (mCSPC) by two randomised controlled trials (RCT), CHAARTED (Chemohormonal Therapy in Metastatic Hormone-sensitive prostate cancer) and STAMPEDE (Systemic Therapy in Advancing and Metastatic Prostate Cancer: Evaluation). Both contributed to a meta-analysis (STOpCaP) which combined aggregate data from 3206 patients with mCSPC demonstrating docetaxel to improve survival by 23%, Hazard Ratio (HR) 0.77; 95% CI 0.68-0.87, p<0.0001) and 4-year survival rate by 9% (range 5-14%).[20-22] These data have changed practice, supported within the UK by a NICE evidence summary and NHS England who recommend that docetaxel is considered for all suitable men with mCSPC.[23,24]

The addition of abiraterone to ADT has also been shown to improve survival and disease control rates in the randomised comparison of abiraterone conducted within STAMPEDE and the co-published LATITUDE trial, a placebo-controlled RCT in an overlapping population of high-risk mCSPC[19,25]. Both trials demonstrated highly significant improvements in survival and disease control rates, which, when combined in a meta-analysis, estimate abiraterone to reduce the risk of death by 38% (HR 0.62; 0.53-0.71; $p=0.55x10^{-10}$); translating into a 14% improvement in 3 year survival rates. Although disease control definitions differ slightly, the effect on clinical or radiological progression free survival (rPFS) was also highly significant (HR = 0.45, 95% CI = 0.40-0.51, p = 0.66 × 10-36)[26]. Therefore, for the first time in over 60 years, the management of mCSPC has been updated and may now include the addition of abiraterone or docetaxel to ADT, see **Figure 2.**

Figure 2: Updated systemic therapeutic options for metastatic prostate cancer

(adapted from Lorente *et al.*[16])



Upfront use of abiraterone or docetaxel given in addition to ADT in the castrate-sensitive setting is the first change to the systemic treatment of metastatic castrate-sensitive prostate cancer in over 60 years.

There are now three potential standard of care (SOC) treatment strategies for men presenting with mCSPC; ADT+docetaxel, or ADT+abiraterone, or in those patients unwilling or unable to receive either, ADT alone. However, the observed degree of clinical benefit is variable, see **Table 3**[20]. Potential benefit must be weighed up against the risk of treatment-related toxicity; often a complex assessment in the predominantly elderly, co-morbid population affected by prostate cancer. Here, trial data likely underestimates risk as variability in treatment tolerance can be expected to be greater in non-clinical trial populations who differ in performance status, age and co-morbid burden. This is supported by emerging data from "real-world" use of docetaxel in the hormone-naïve setting, where the reported frequency of neutropenic sepsis is 20% compared with 2-15% in trial populations[20,21,27-29]. In addition, abiraterone and docetaxel have different therapeutic mechanisms of action, leading to the hypothesis that differences in prostate cancer biology may predict differential treatment effects.

Table 3: Metastatic survival outcomes in STAMPEDE docetaxel comparison

	ADT	ADT + Docetaxel
5-year survival	39%	50%
Median OS	42 months	60 months
Calculated IQR	*68 months* (23 – 91 months)	*76 months* (27-103 months)

Key: IQR Interquartile range, OS overall survival
NB: At the time of the primary analysis of the STAMPEDE abiraterone comparison the median OS is unreported. There is insufficient follow up to report 5 year survival.

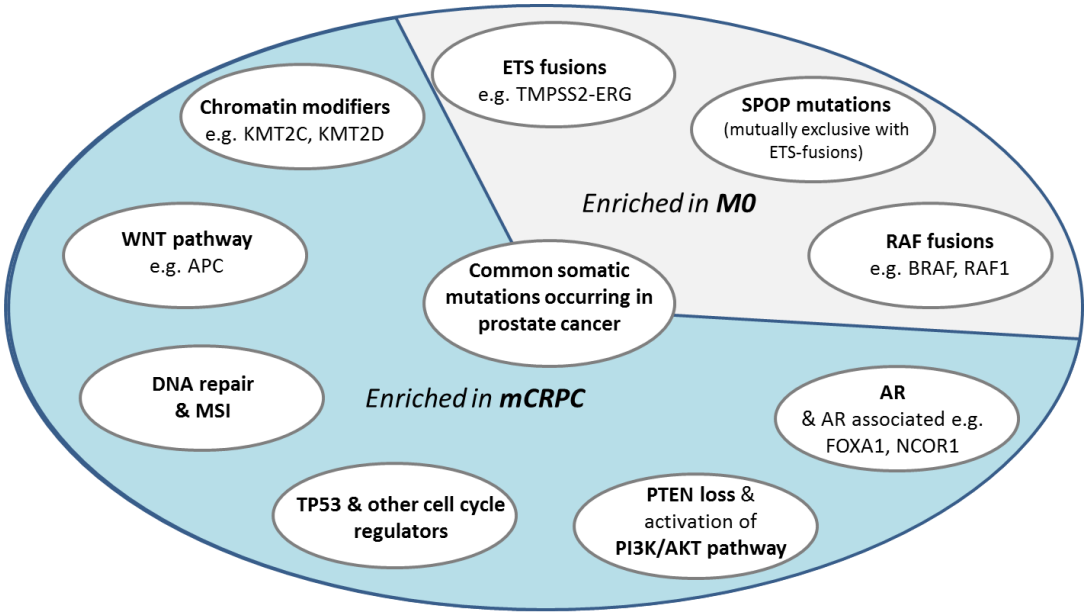
1.2 Molecular basis of prostate cancer

Improvements in the understanding of the molecular basis of PCa have revealed genetic diversity that may help explain the observed clinical variability. The Cancer Genome Atlas (TCGA) has comprehensively profiled treatment-naïve non-metastatic (M0) prostate cancers through sequencing prostatectomy samples. Their findings have demonstrated copy number alterations, recurrent somatic point mutations and oncogenic structural DNA rearrangements, termed chromoplexy, are all common genomic feature of M0 disease[30,31]. Overall, the most frequent alterations, occurring in 40-60%, are fusions of androgen-regulated promoters such as transmembrane protease, serine 2 (*TMPRSS2*) with erythroblast transformation-specific (ETS) transcription factors such as *ERG*, *ETV1* and *ETV4*[30,32]. Other fusions are observed, although less frequently, such as those involving B-

Raf proto-oncogene (*BRAF*) and the Ras family GTPase (*RAS*), identified in 1-2%[30,33]. Sequencing the whole genome enables large chromosomal deletions or amplifications to be detected, termed somatic copy number alterations (CNA). These frequently occur and commonly affected regions include chromosome 8, spanning the proto-oncogene c-myc and chromosome 10 affecting phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*)[30]. Recurrent somatic mutations are also observed; the most frequently affected gene is Speckle-Type POZ Protein (*SPOP*), mutated in 6-13% of primary prostate cancers and mutually exclusive with ETS-fusions[34]. Other frequently mutated genes include the androgen receptor associated gene Forkhead Box A1 (*FOXA1*), tumour protein p53 (*TP53*) and chromatin modifiers such as Chromodomain-helicase-DNA-binding protein 1 (*CHD1*)[30], see **Figure 3**.

The genomic profile of metastatic PCa has been studied most in the setting of advanced or fatal CRPC, with the majority of data coming from trial cohorts enrolled in precision medicine initiatives or rapid autopsy programmes[30,31,34-39]. When compared with M0 treatment naïve disease, both the mutational burden and copy number burden is increased[33]. The accumulating genetic aberrations correlate with tumour progression and likely further drive intra-patient heterogeneity. There is a relative paucity of sequencing data acquired in men presenting with mCSPC. The MSK-IMPACT cohort included the most cases (n=140), however possibly reflecting differences in PSA screening practices in the US, half of cases were being treated for relapsed disease having originally presented with localised disease which was previously radically treated[33]. In contrast, over 90% of men enrolled with mCSPC in STAMPEDE present with *de novo* metastatic disease. It has been suggested that the biology of this aggressive high-risk phenotype may be more similar to mCRPC than the more indolent localised disease profiled through sequencing prostatectomy cohorts. To test this hypothesis, genomic data acquired from men with *de novo* metastatic disease is required and this is a pre-requisite to evaluating potential molecularly-selected treatments in this setting.

Figure 3: Frequent mutations occurring in prostate cancer



Frequent mutations occurring in prostate cancer: Several somatic (acquired) mutations are recognised affecting multiple pathways. In metastatic castrate resistant disease, the most frequently mutated gene is the androgen receptor (*AR*), aberrant in up to 70%, consistent with the emergence of androgen-independent AR-transcriptional activity, a well-recognised feature of progression to CRPC[39]. At the pathway level, PI3K, DNA repair, cell cycle and Wnt pathway aberration are all observed and are more frequent in mCRPC compared with localised, treatment-naïve prostate cancer[33]. Activation of the PI3K pathway is seen in around 50%, which may occur through biallelic loss of PTEN or activating mutations, fusions or copy number amplification of *PI3K* subunits or *AKT*. Dysregulation of the cell-cycle pathway is predicted in around 20% of mCRPC as a result of *RB1* loss, or amplification of CDK kinases including *CCND1*, *CDK4*. Whilst mutations and copy number loss of genes involved in DNA repair, specifically homologous recombination, has been shown to occur in between 15-23% of mCRPC; affected genes include *BRCA2*, *CDK12*, *FANCA*, *Rad51* paralogues and *ATM*[39,40].

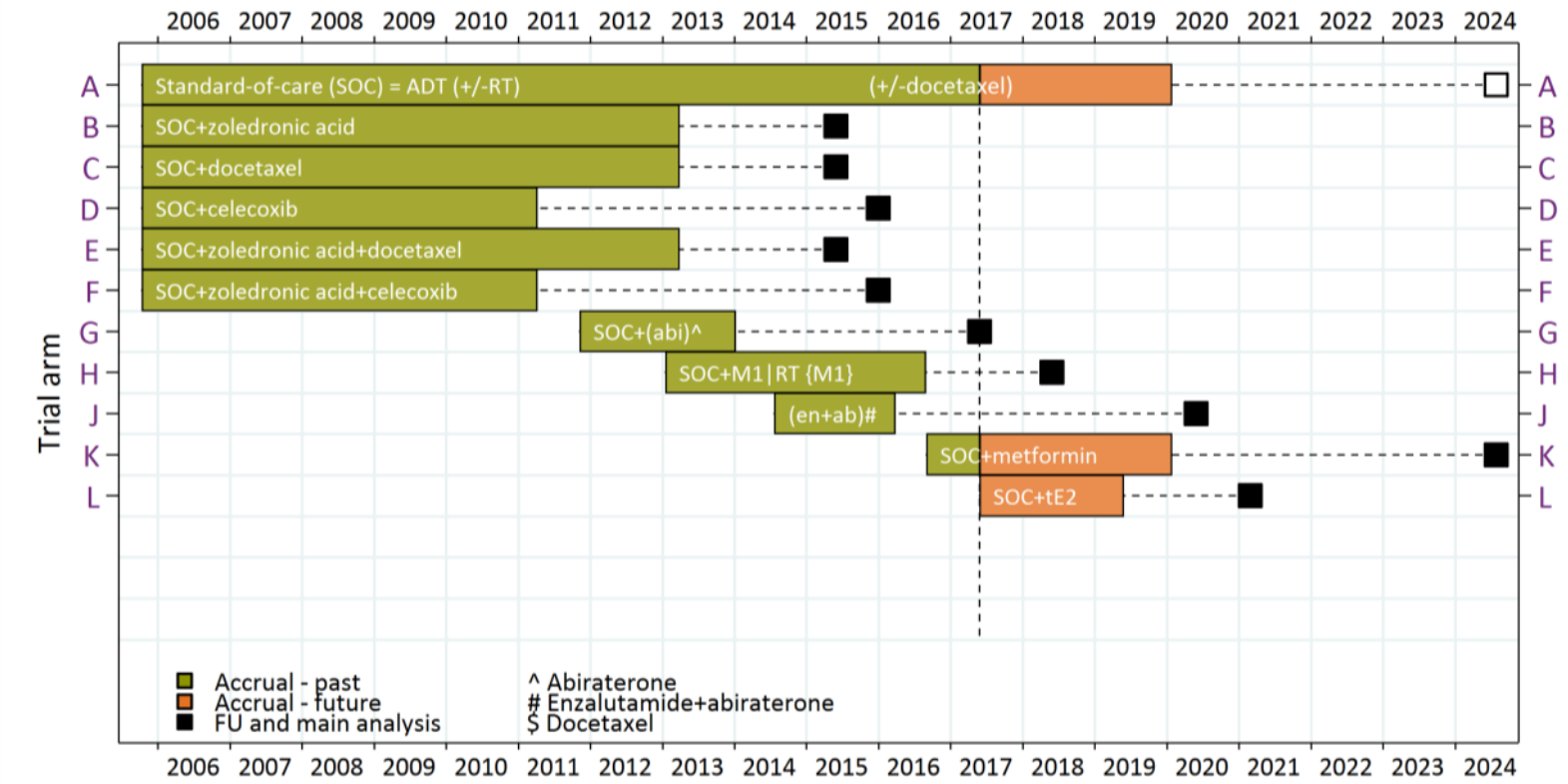
Key: *AR*, Androgen receptor; *ATM*, Ataxia telangiectasia mutated gene; *BRAF*, v-Raf murine sarcoma viral oncogene homolog B; *BRCA2*, BReast CAncer susceptibility gene 2; *CDK*, Cyclin dependent kinases; *CCND1*, Cyclin D1; *CDK4*, cyclin dependent kinase 4; *CDK12*, cyclin dependent kinase 12; CRPC, Castrate-resistant prostate cancer; DNA, Deoxyribonucleic acid; *NCOR1*, Nuclear receptor co-repressor 1; *PI3K*, Phosphatidylinositol-4,5-bisphosphate 3-kinase; *PTEN*, phosphatase and tensin homolog deleted on chromosome 10; *RB1*, Retinoblastoma 1.

1.3 STAMPEDE clinical trial

1.3.1 Trial design

STAMPEDE is a large ongoing RCT evaluating therapeutic strategies for the management of men with high-risk localised or mCSPC (www.stampedetrial.org). The trial employs a multi-arm multi-stage (MAMS) platform design: multi-arm because many treatment approaches can be tested simultaneously, and multi-stage because pre-specified interim analyses can be used to stop recruitment early to arms showing insufficient evidence of activity[41]. Data from all stages is included in the final analysis of efficacy, powered on the primary outcome; OS. The trial opened in 2005 with five comparisons evaluating the efficacy of adding therapies to ADT, the then SOC (**Figure 4)**. The additional treatments assessed were docetaxel chemotherapy, the bisphosphonate zoledronic acid (ZA) and celecoxib, a cox-2 inhibitor. These three treatments were evaluated alone and in combination, to give a total of 5 research arms (B-F); since referred to as the original comparisons. One of the advantages of the MAMS platform design is that new research arms can be incorporated within a single protocol, utilising the established trial infrastructure and thus enabling rapid activation of recruitment to new research questions[42]. Since the start of the trial, a number of new research arms have been added to STAMPEDE to evaluate: abiraterone (arm G); prostate RT for patients with newly-diagnosed metastatic disease (arm H); enzalutamide given in combination with abiraterone (arm J); metformin (arm K), an anti-diabetic medication and transdermal oestradiol (arm L), as an alternative approach to ADT. To date over 10,000 men have enrolled and the STAMEPDE trial remains the largest ever RCT in this disease setting.

Figure 4: STAMPEDE overview: arms open to recruitment over time (2005-current)



STAMPEDE Trial overview: The STAMPEDE trial uses a MAMS design to conduct multiple randomised comparisons between research arms and a shared control arm (A). Research arms B-F are referred to as the original comparisons and together with research arm G have reported the primary analysis. Comparisons made between contemporaneous A, H and J are yet to be reported. The dashed line highlights the latest research arm to be added into the platform, arm L activated in June 2017. As of May 2018 the current recruiting arms are A, K and L.

1.3.2 Summary of STAMPEDE results to date

Docetaxel-containing comparisons (research arms A-C, A-E)

The primary analyses of the original comparisons are published. As summarised in Section 1.1.2 the docetaxel results have contributed to a change in the SOC, supported by data from the CHAARTED trial[21]. The STAMPEDE docetaxel comparison (control and arm C) was comprised of 1,776 men with either high-risk locally advanced disease (~40%) or metastatic disease (~60%) and demonstrated that the addition of docetaxel improved survival by 22%, with a 10 month improvement in median survival from 71 to 81 months; HR 0.78; (95%CI 0.66- 0.93) p=0.006. No evidence of heterogeneity was seen by metastatic status, however survival data is more mature in the poor-prognostic metastatic subgroup (n=1086) in whom the addition of docetaxel was shown to result in a 15 month improvement in median survival (45 to 60 months). Although survival data remains immature in the M0 subgroup, a significant improvement in failure-free survival (FFS) is observed; FFS is defined as the first of radiological, clinical or PSA progression or death from PCa (HR 0·60, 95% CI 0·45–0·80; p=0·283 × 10^{-3}). Again, no heterogeneity in treatment effect is observed by metastatic status and the effect on FFS in the metastatic subgroup is consistent (HR 0·61, 95% CI 0·53–0·71; p=0·283 × 10^{-10}). Docetaxel was also evaluated in addition with ZA (comparison of control arm with research arm E). The addition of ZA was not shown to confer any additional benefit in FFS or survival over docetaxel, consistent with the results of the ZA comparison, which showed no benefit[20]. See **Table 4** for a summary of all trial data evaluating docetaxel in CSPC.

Table 4: Summary of survival data from trials evaluating docetaxel in mCSPC

Trial acronym	Patient group	Randomised comparison (n)	Treatment detail	Summary of efficacy data (OS)
GETUG-15[27]	mCSPC	385	• ADT • ADT + docetaxel (up to 9 cycles)	Median OS 58.9 (95%CI 50.8-69.1) vs. 54.2m (95%CI 42.2-NR) HR 1.01 (95%CI 0.75-1.36) p=0.955
CHAARTED[21]	mCSPC	790 High-volume M1 n=513	• ADT • ADT + docetaxel (up to 6 cycles)	***Overall*** Median OS 57.6m vs. 44.0m; HR 0.61 (95%CI 0.47-0.80) p<0.001 ***High-volume M1 subgroup*** Median OS 49.2m vs. 32.2m; HR 0.60; 95% CI 0.45-0.81; p<0.001
STAMPEDE[20]	mCSPC and high-risk M0 CSPC	Total 1776 M1 subgroup n=1086	• ADT • ADT + docetaxel+ prednisolone (up to 6 cycles)	***Overall*** Median OS 81m (IQR 41-NR) vs 71m (IQR 32-NR) HR 0.78 (95%CI 0.66-0.93) p=0.006 ***M1 subgroup*** Median OS 60m (IQR 27-103) vs. 45m (IQR 23-91); HR 0.76 (95%CI 0.62-0.92) p=0.005
STOpCaP meta-analysis[22]	mCSPC and high-risk M0 CSPC	M1 n=3206 M0 n=3978	• ADT • ADT + docetaxel (6-9 cycles) +/- prednisolone	***M1 subgroup*** HR 0.77 (95% CI 0.68-0.87) p<0.0001) Improvement in 4-year survival rates 40-49% (range 5-14%) *M0* HR 0.87 (95% CI 0.69-1.09) p=0.218

Summary of docetaxel efficacy data: Both GETUG-15 and CHAARTED trials restricted recruitment to patients with M1 disease. CHAARTED performed a subgroup analysis by disease volume; high-volume M1 was defined by the presence of visceral metastases or four or more bone lesions with ≥1 outside the spine or pelvis. Only STAMPEDE recruited men with M1 or high-risk M0 disease.

Key: ADT, androgen deprivation therapy; CSPC, castrate-sensitive prostate cancer; HR, Hazard ratio for overall survival; m, months; M0,non-metastatic; M1, metastatic; OS, overall survival.

Celecoxib-containing comparisons (research arms A-D, A-F)

In contrast to the docetaxel data, the results of the celecoxib-containing comparisons are intriguing and hypothesis generating rather than practice changing[43]. As afforded by the MAMS trial design, STAMPEDE has evaluated celecoxib alone (arm D) and in combination with ZA (arm F). In total, 1,245 men enrolled between October 2005 and April 2011 were randomised 2:1:1 to receive SOC (ADT+/- pelvic radiotherapy), or SOC + celecoxib or SOC + celecoxib + ZA. Celecoxib treatment was given orally 400mg BD for 1 year; ZA was given monthly for a total of 2 years using a standard dose of 4mg. Of note, the duration of celecoxib was reduced from 2 years to 1 year following regulatory advice sought by the STAMPEDE Trial Management Group (TMG), prompted by the withdrawal of the cox-2 inhibitor rofecoxib. In 2011, recruitment was halted to both the celecoxib-containing research arms due to insufficient activity demonstrated for the intermediate outcome of FFS (pre-defined target HR 0.92).[44] Lifelong follow-up continued and survival data was presented shortly after the reporting of the primary efficacy analyses of the remaining original comparisons to which recruitment had continued. Overall, no survival benefit is shown for either celecoxib, ZA, or the combination. However, in a pre-planned subgroup in men with M1 at presentation (n=567) the combination of celecoxib-ZA is shown to improve survival by 22% (HR=0·78, 0·62-0·98; p=0·033)[20,44]. This is despite no effect being shown in this subgroup when either treatment is given alone, see **Table 5.**

Table 5: Results of the STAMPEDE celecoxib-containing comparisons

Patient group	**Comparison made between arm A (control) and the following research arms**		
	Research arm D	*Research arm E*	*Research arm F*
	ADT + celecoxib[43]	**ADT + ZA[20]**	**ADT + celecoxib + ZA[43]**
M1 & high-risk M0	No benefit HR 0.98 (95% CI 0.80-1.20) P = 0.847	No benefit HR 0·94 (95% CI 0·79–1·11) p=0·450	No benefit HR 0.86 (95% CI 0.70-1.05) P =0.130
M1 subgroup	No benefit HR, 0.94 (95% CI 0.75-1.18) P = 0.602	No benefit HR 0.93 (95% CI 0·77–1·11) p=0·416	Survival benefit observed HR 0.78 (95% CI 0.62-0.98) p=0.033)

Abiraterone comparison (research arms A-G)

The results of the STAMPEDE abiraterone comparison also show improved outcome with an intensified upfront treatment strategy. 1917 men with high-risk M0 or metastatic castrate-sensitive prostate cancer were randomised 1:1 to receive ADT or ADT + abiraterone 1000mg BD + prednisolone 5mg daily. Treatment duration was dependent on disease stage and treatment intention. For patients with M0 disease receiving radical prostate RT, abiraterone was to continue for 2 years or until progression, whichever came first. For all others, abiraterone was to continue until progression, defined as a PSA rise, symptomatic or radiological or another treatment for progressive disease was started. In keeping with the approach adopted by the two pivotal trials in CRPC (COU-AAA-301 and COU-AAA-302), abiraterone could continue until all three types of progression had occurred, if judged by the treating clinician to be in the patients' best interest [9,10].

Overall, the use of abiraterone with ADT + prednisolone improved the time to treatment failure by 71% and OS by 37%, compared with ADT alone. The treatment effect was consistent in both metastatic and M0 subgroups; although survival data remains immature in the latter. Within the metastatic subgroup the estimated survival effect is HR 0.61 (95% CI 0.49-0.75), with an even larger effect observed on FFS; HR 0.29 (0.25-0.34); p 0.38 x10-61). These data are consistent with the results of the LATITUDE trial which demonstrated a comparable OS improvement: HR 0.62 (0.51-0.76) p<0.001) and both contributed to a meta-analysis (STOpCaP), see **Table 6** [25,26].

There have been two recent analyses which attempt to address the question of which treatment, abiraterone or docetaxel can achieve the greatest survival improvement. A network meta-analysis using aggregate data would suggest superior survival gains with abiraterone (HR = 0.61, 95% CI 0.51-0.76) over docetaxel (HR = 0.74, 95% CI 0.65-0.85)[45]. However an opportunistic direct comparison is possible within STAMPEDE due to the overlap in the accrual to both docetaxel and abiraterone containing comparisons between November 2011 and March 2013. In contrast, this provides no strong evidence for a survival advantage with one treatment compared with the other. However, measures of disease control rates such as FFS and PFS which excludes PSA-driven failure, all favour abiraterone[46]. The latter result provides strong evidence that abiraterone given in the castrate-sensitive state successfully delays the progression to CRPC, but the failure of this to translate into a survival advantage over docetaxel may reflect the negative impact abiraterone use may have on access and response to second-line therapies. For example,

currently within the UK, prior abiraterone is a contraindication to accessing funding to enzalutamide in CRPC due to evidence of cross-resistance[47]. In summary, there is no strong evidence that either docetaxel or abiraterone is superior based on this direct comparison; the underpowered comparison of prostate cancer specific survival would suggest they are equivalent HR 1.02 (0·70-1·49); as such, both remain SOC options. Therefore the challenge now facing clinicians, patients and healthcare funders is to determine which individuals are most likely to benefit from each treatment strategy.

Table 6: Summary of abiraterone efficacy data in mCSPC

Trial acronym	Patient group	Randomised comparison (n)	Treatment detail	Summary of efficacy data (95% CI)
STAMPEDE	M1 and high-risk M0 CSPC	1917 randomised 1:1 (M0 915; M1 1002)	• ADT • ADT + abiraterone + prednisolone	Overall: • OS HR 0.63 (0.52-0.76); p<0.001 • Improvement in 3-yr survival from 76% to 83% By metastatic subgroups: • M1: OS 0.61 (0.49-0.75), FFS 0.31 (0.26-0.37) • M0: OS 0.75 (0.48-1.18), FFS 0.21 (0.15-0.31)
LATITUDE	Metastatic CSPC (high-risk)	1199 randomised 1:1	• ADT + dual placebo • ADT + abiraterone + prednisolone	Overall (Dual primary endpoints): • OS HR 0.62 (0.51-0.76) p<0.001 • rPFS HR 0.47 (0.39-0.55) p<0.001
STOpCaP meta-analysis[22]	Metastatic CSPC	2201	• ADT +/-placebo • ADT + abiraterone + prednisolone	Overall: • Overall survival HR: 0.62 (0.53-0.71); p=0.55 x10-10 • Improvement in 3 year survival from 55% to 69%

Summary of abiraterone efficacy data: STAMPEDE recruits both M1 and M0 CSPC. The treatment duration is up to 2 years or progression in patients with M0 disease who receive radical RT. Otherwise, treatment was planned to continue until all three types of progression (PSA, symptomatic and radiological) occurred, or second-line treatment was started. LATITUDE recruited an overlapping population defined as high-risk mCSPC by the presence of 2 out of 3: Gleason ≥8, at least 3 bone lesions and the presence of measurable visceral disease.

Key: ADT, androgen deprivation therapy; HR, Hazard ratio; HNPC, Hormone-naïve prostate cancer; M0, non-metastatic; M1, metastatic; OS, overall survival

1.4 Identified research priorities

Acquiring data that can inform treatment selection in mCSPC is now a priority to ensure the recent evidence of benefit shown for intensified treatment strategies translates to the best individual outcome. This is the concept of personalised medicine, an area where prostate cancer has lagged behind other solid tumour types. Factors likely contributing to this include the predominance of the AR pathways and the almost universal initial response to ADT. The development of predictive genomic biomarkers is also made more challenging by tumour tissue availability, typically limited to small prostate core biopsies, whilst the predominance of bone metastases means sampling metastatic disease is technically difficult, requiring specific protocols if sufficient concentration of high quality DNA are to be obtained[48]. Added to this is the lack of validated intermediate clinical endpoints, meaning that prognostic and predictive questions are reliant on analyses of OS, increasing the cost and time of data acquisition. However, in anticipation that the trend will continue for the greatest absolute benefit of effective treatments to be seen when used at first presentation, the need for improved tools to risk-stratify, prognosticate and predict treatment response and/or toxicity is only set to increase.

Personalised medicine approaches may improve outcome through identifying patients who have a differential treatment response and providing these groups are not overlapping, outcomes in the group overall will improve as a result of optimised treatment. Whilst at a societal level, through reduced use of therapies in populations predicted to benefit least, this approach offers a more efficient allocation of healthcare resource. Paired to the concept of personalised medicine, is the term biomarker, used to define a measurable characteristic that is an indicator of a process, be it biological, pathogenic or response to a therapeutic intervention[49]. The implementation of a personalised approach is reliant on biomarkers to identify and characterise patient groups in whom specific treatment strategies should be evaluated. In this thesis I will explore how the development of biomarkers in the setting of mCSPC can be best achieved through analyses of existing and future data collected as part of the STAMPEDE trial.

1.5 How can STAMPEDE data inform treatment selection?

Biomarker development can be facilitated through additional analyses conducted in parallel or as part of clinical trial protocols. As described in the Cancer Research UK (CRUK) prognostic and predictive biomarker roadmap, see **Figure 5**, correlative analyses using

biological samples obtained from clinical trial cohorts provide multiple opportunities to support biomarker development[50]. These include enabling retrospective analyses of biomarker status correlated with clinical outcome and testing of hypotheses to explain heterogeneity in treatment response. Samples may also be used to optimise biomarker assays, or estimate prevalence in the population of interest. Access to sample collections and correlative clinical data is a valuable resource which can support external validation. Finally, the clinical utility of validated biomarkers can be confirmed through protocols in which biomarker status defines randomisation or patients are randomised to the strategy of biomarker-directed therapy vs. non biomarker-directed treatment.

All participants joining the STAMPEDE trial have been asked to consent to donate remaining diagnostic tissue e.g. formalin-fixed paraffin embedded (FFPE) prostate core biopsies for use in separate ethically approved translational protocols. As mature outcome data is now known for several randomised comparisons, correlative retrospective analyses are possible. As an adaptive trial platform, the protocol can also be amended to address biomarker-driven questions, acquiring prospective data to evaluate this approach.

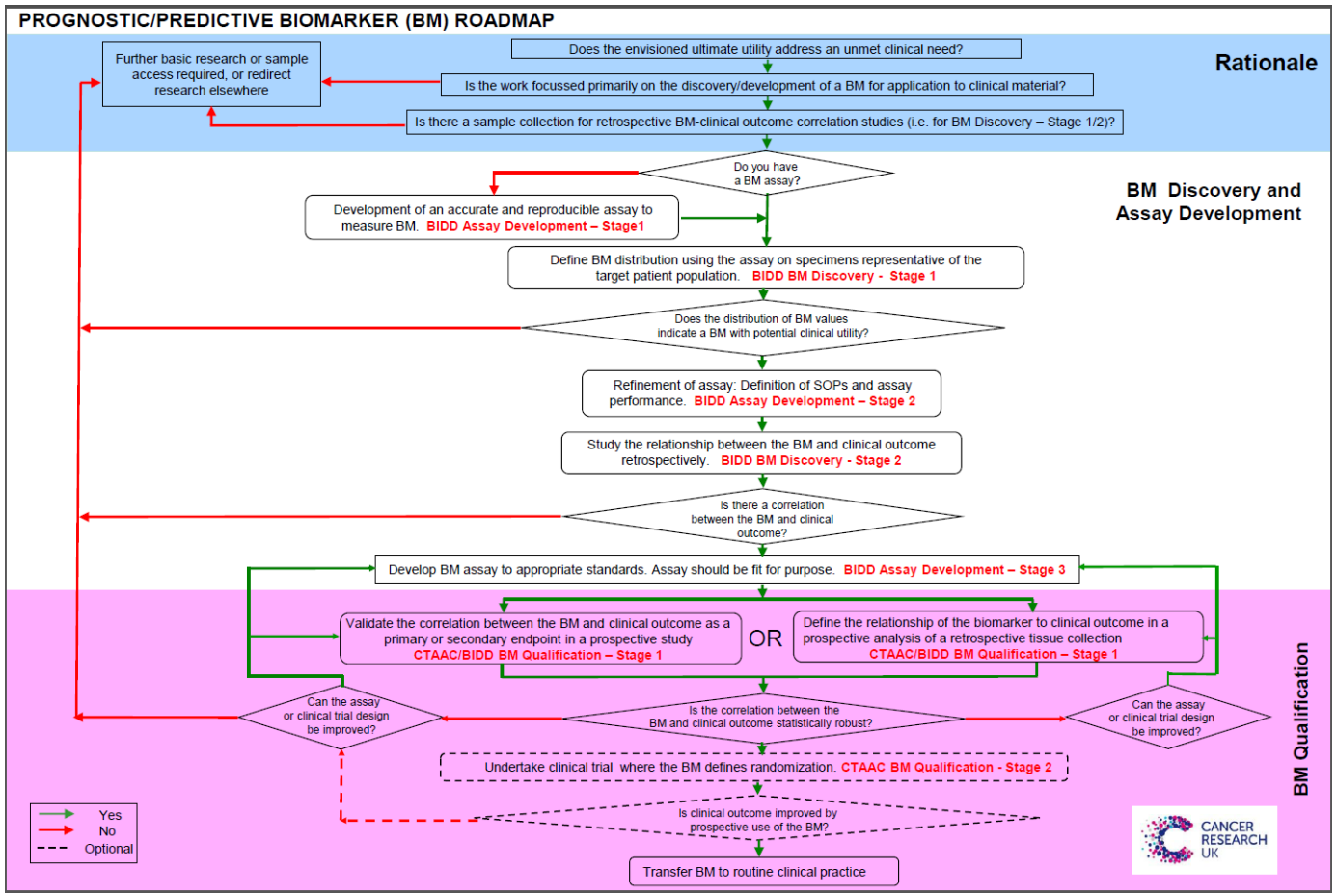
1.5.1 Seek support and understanding of variable treatment effects

The results of the celecoxib-containing comparisons provide evidence of unexpected heterogeneity in treatment effect. An additive effect for the combination of celecoxib-ZA is observed, selectively beneficial in men with metastatic disease. The results are clinically significant with a 22% improvement in OS and evidence of good tolerability; the proportion of patients reporting a Common Terminology Criteria for Adverse Events version 3.0 (CTCAE) toxicity grade ≥3 was comparable in all groups: control-SOC alone (36%), SOC + celecoxib (33%) and SOC + celecoxib + ZA (32%). The magnitude of benefit is similar to that observed for docetaxel; however in contrast, these data have not translated into a change in practice. This is likely due to the lack of supporting clinical data and uncertainty around the mechanism of action sufficient to explain the additive effect observed between celecoxib-ZA and why this should be selective to metastatic disease.

One approach to biomarker identification is through seeking to identify and understand heterogeneity in therapeutic responses. This strategy may enable characterisation of a subpopulation who may benefit from a particular treatment. Further investigation, particularly involving molecular characterisation, may in turn reveal distinct mechanisms of response and support biomarker development. This is the rationale behind the study of

exceptional responders supported by several precision medicine intiatives[51]. Furthermore there are several examples of targeted therapeutic strategies, initially evaluated in unselected populations but now licenced in biomarker-defined groups, characterised through parallel translational research, such as cetuximab in Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type colorectal cancer or epidermal growth factor receptor (EGFR) inhibitors in EGFR-mutant non-small cell lung cancer (NSCLC)[52-54]. I will seek to contextualise and understand the results of the celecoxib-ZA comparison and explore biological hypotheses to explain the observed selective effect in mCSPC.

Figure 5: Cancer Research UK Biomarker Roadmap



Cancer Research UK Biomarker Roadmap: Outlines the multiple ways in which clinical trials can support biomarker development through assay refinement, retrospective biomarker-clinical outcome correlation studies, prospective analyses and qualification studies whereby biomarker status determine eligibility for randomisation. Clinical trials using adaptive protocols may support multiple stages.

1.5.2 Improved risk-stratification

As PCa predominantly affects older men, treatment paradigms emphasise the need to balance relative life expectancy with the risk of lethal disease[55]. Over half of men with newly diagnosed PCa are aged 70 years and over and almost all have co-morbidities that can be anticipated to impact on treatment tolerance to some degree[1,55] Accurate identification of high-risk cancers may provide the justification for intensified therapeutic strategies and ensure optimal use without compromising quality of life through risking unnecessary toxicity. Prognostic biomarkers may aid this assessment and inform clinical trial design, through identifying patient subgroups with a similar risk profile in whom to evaluate a specific treatment strategy[56]. Through characterising prognostic biomarkers, insights into the biological basis of heterogeneity in outcome may also be gained. The increasing number of available therapies means the need for improved prognostic tools continues to grow.

Through using PSA measurements collected as part of the STAMPEDE protocol I will evaluate two proposed on treatment biomarkers with the aim of improving risk stratification pre and post docetaxel use in the castrate-sensitive setting. PSA response is proposed as an early prognostic indicator able to assess initial ADT response, with the magnitude of response hypothesised to correlate with improved survival outcome. This can be used to identify patients at low risk in whom the toxicities of docetaxel may be avoided. Secondly, I will evaluate if PSA nadir, defined as the lowest PSA on treatment is prognostic of survival when assessed on completion of docetaxel. It is hypothesised that high PSA nadir, shown to be prognostic in mCSPC treated with ADT alone, may identify a high-risk group in whom further intensified treatment strategies should be considered. Several ongoing trials are evaluating the combination of ADT + docetaxel + AR-targeted therapy such as abiraterone or enzalutamide (see **Table**). Whilst it is yet to be shown if the benefits of docetaxel and abiraterone will prove additive, many anticipate that this further intensified treatment strategy will be more effective. However, given the associated additional cost and toxicity, it is unlikely that this will be considered suitable for all, emphasising the role of improved risk-stratification at this time point.

1.5.3 Identify potential predictors of response

The knowledge of the genomic landscape of PCa has increased rapidly within the last decade due to advances in high-throughput sequencing technologies. This provides both the means and the rationale to evaluate molecularly-selected treatment strategies within a subset of patients characterised by a putative predictive biomarker.

The results of the Trial of PARP inhibition in Prostate Cancer (TOPARP) study provide proof-of-concept of this approach in mCRPC. This phase 2 trial recruited 50 heavily pre-treated patients with mCRPC; all had received docetaxel, 49/50 had received abiraterone or enzalutamide and over half had received cabazitaxel. Olaparib, a PARP inhibitor (PARPi), was shown to be selectively active in mCRPC with defects in genes involved in homologous recombination DNA repair pathway. Biomarker assessment involved targeted next-generation sequencing (tNGS) of mandated fresh tumour biopsies, obtained prior to trial entry. Response was defined using a composite endpoint that included any of the following: radiological response, according to Response Evaluation Criteria In Solid Tumours (RECIST) criteria; PSA reduction ≥50%, or confirmed circulating tumour cell (CTC) count conversion from ≥5 per 7.5ml to <5 per 7.5ml. Overall, responses were seen in 88% (14/16 cases) of cases associated with detectable homologous recombination deficiency (HRD). The median duration of treatment was 9 months with 4/16 patients continuing olaparib for more than 12 months. Crucially, only 6% (2/33) patients defined as biomarker negative responded, suggesting a high specificity of the tNGS panel[57].

This evidence of selective clinical benefit provides the motivation to profile mCSPC, aiming to determine if HRD is present at the first presentation of metastatic disease, thus providing a rationale to evaluate PARPi in this setting. As has been seen to date, the greatest absolute impact on outcome may be seen when effective treatments are used earlier and it is hypothesised that this may be particularly relevant to therapeutic strategies that seek to exploit defective mechanisms of DNA repair. The presence of which can be expected to drive genomic instability and the development of genetic heterogeneity, the latter can thwart targeted medicine approaches due to the risk of co-existing sub-clonal mutations that may confer treatment resistance[58,59].

The adaptive STAMPEDE platform has been shown to be amenable to evaluating questions in a subset of the broader eligible population, the best example of this being the assessment of prostate RT in men with newly diagnosed metastatic disease. 2061 patients

were recruited between January 2013 and September 2016 to this comparison, made between patients allocated to research arm H and comparable controls. Accrual was sustained alongside other questions that addressed treatments relevant to the broader population of metastatic and high-risk M0 disease. One of the perceived challenges of addressing biomarker-selected questions is the anticipated low prevalence, leading to a high screening burden. Therefore it is proposed that the most efficient route to evaluation may be to further amend the STAMPEDE trial; allowing recruitment to both biomarker-selected and unselected comparisons within one inclusive platform protocol.

There are several recognised challenges when considering the implementation of a biomarker-selected randomised comparison in this setting. Firstly, in keeping with the pragmatic approach that has helped ensure the STAMPEDE trial can successfully recruit at both academic and district general hospitals, the aim would be to avoid additional tumour sampling. Therefore implementation of prospective biomarker screening would rely on rapid retrieval and centralised testing of remaining diagnostic tissue, typically prostate core biopsies stored as FFPE blocks. Through undertaking a retrospective analysis of clinically representative samples, I will assess the test performance and thus the feasibility of this approach. I will also aim to pilot several of the operational changes required, including establishing collaborations with a biorepository and sequencing laboratories, as well as testing new processes to track samples and securely transfer genetic data. Finally, I will aim to estimate the prevalence of HRD in mCSPC, as this determines the number needed to screen, accrual rate and trial duration so is considered the most important parameter when evaluating the feasibility of this approach and defining the optimum trial design.

1.6 Summary of research objectives

In this thesis I will aim to improve outcomes for patients with mCSPC through a deeper understanding of the results generated from STAMPEDE so far and through genomic profiling aiming to evaluate if it is feasible to evaluate upfront PARP inhibition in this disease setting.

First, I will seek to contextualise and understand the selective beneficial effect of the combination of celecoxib-ZA through undertaking a systematic review and, where possible, a meta-analysis aiming to evaluate if there is external clinical data to support the observed clinical efficacy. In addition, relevant clinical and pre-clinical data will be reviewed with the

aim of generating biological hypotheses that may explain the observed additive effect of this therapeutic combination, and why this should be selective to metastatic disease.

Secondly, I will explore the relationship between PSA and OS aiming to evaluate two PSA-based outcomes as potential prognostic biomarkers capable of risk-stratifying patients and informing treatment selection in the castrate-sensitive setting. I will explore if the magnitude of early PSA response on ADT is associated with survival, testing the hypothesis that a large PSA response may identify a good prognostic subgroup. Secondly, I will evaluate the relationship between PSA nadir on docetaxel and OS as a proposed on treatment biomarker that may be able to identify patients that remain at high-risk in whom intensified therapeutic strategies in the castrate-sensitive setting should be evaluated.

Finally, through collaboration with industry partners, I will assess the feasibility of implementing prospective molecular biomarker screening within STAMPEDE in order to identify a population hypothesised to benefit from PARP inhibition. Through additional exploratory analysis of the sequencing data I will aim to explore the broader genomic profiles to better understand whether future genomic biomarker-treatment pairings may be evaluated through further adaptation of the STAMPEDE trial platform.

Chapter 2 Contextualising the STAMPEDE celecoxib and zoledronic acid results

2.1 Introduction

The aim of this systematic review and meta-analysis is to contextualise the results of the STAMPEDE celecoxib-containing comparisons. Overall, no survival benefit was seen for either celecoxib, ZA, or the combination. However, in a pre-planned subgroup analysis in men with metastatic disease at presentation (n=567) the combination of celecoxib-ZA is shown to improve survival by 22% (HR=0·78, 0·62-0·98; p=0·033)[18,40]. This clinically and statistically significant finding is consistent with a heterogeneity in treatment effect according to baseline metastatic status (p=0.072). Furthermore, no evidence of benefit was observed when either celecoxib or ZA were added alone, consistent with an additive effect for the combination, see **Table 5**.

In 2003 when the STAMPEDE trial was in development, cox-2 inhibitors were novel agents under evaluation in multiple indications. However by the time of data reporting in 2016, the perception and use of cox-2 inhibitors was very different and this result was unexpected. Despite the relative survival gain being comparable with the practice changing results of the docetaxel comparison, see **Table 5**, **Figure 6** and **Figure 7**, these data are hypothesis generating as opposed to practice changing, largely due to the absence of supporting data. I will determine if this intriguing finding has been identified in any other clinical data and explore hypotheses seeking to understand it further.

Figure 6: Survival in metastatic subgroup in docetaxel comparison

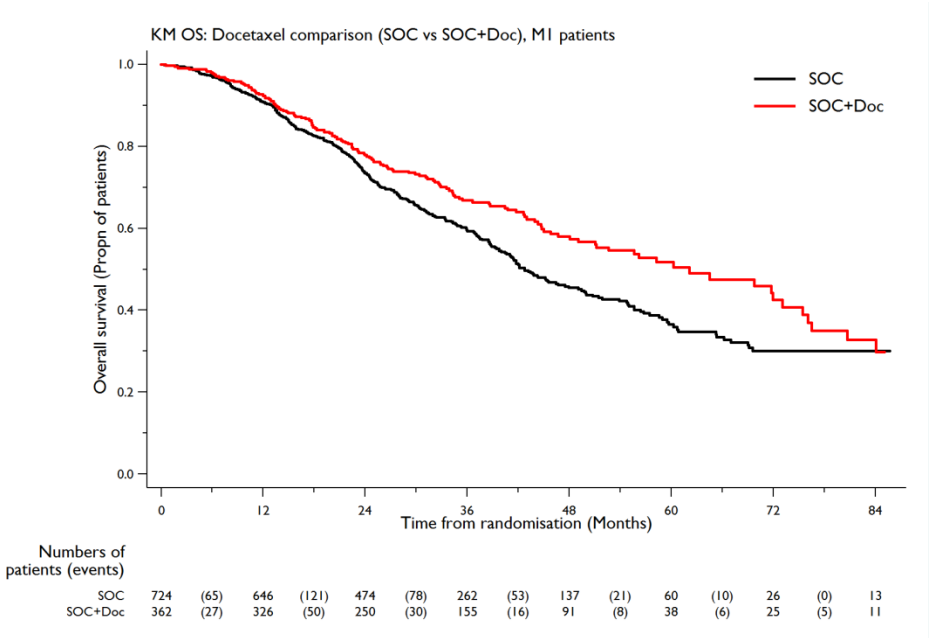


Figure 7: Survival in metastatic subgroup in celecoxib-ZA comparison

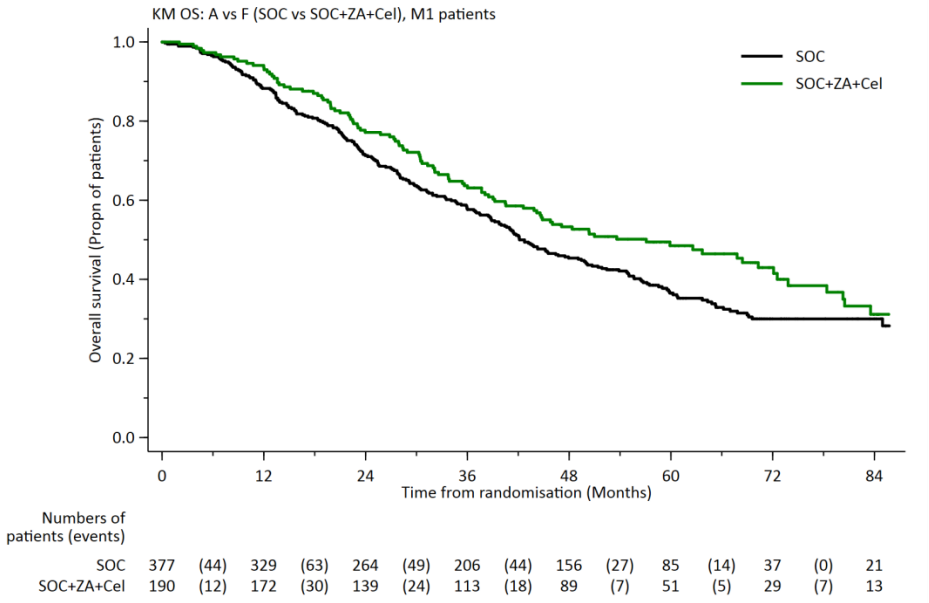


Figure 6 shows the difference in overall survival between men (n=1089) with metastatic castrate-sensitive prostate cancer randomised between control ADT alone and ADT + docetaxel; HR 0.76 (95%CI 0.62-0.92) p=0.005. **Figure 7** shows the difference in overall survival between men (n=567) with metastatic castrate-sensitive prostate cancer randomised between control (ADT alone) and celecoxib + zoledronic acid; HR 0.78 (95% CI 0.62-0.98) p=0.033).

2.1.1 Rationale to evaluate cox-2 inhibitors as anti-cancer agents

Cox-2 inhibitors are non-steroidal anti-inflammatory drugs (NSAID) developed initially as analgesics. NSAIDs all act to inhibit the cyclooxygenase (cox) enzyme family, also known as prostaglandin endoperoxide synthetase (PTGS), reducing the production of prostaglandins, prostacyclin and thromboxanes, which mediate signal-transduction pathways modulating cellular growth, adhesion and proliferation[60]. Cox-1 is widely expressed in normal tissue and is thought to have a "housekeeping" role, which includes mediating prostaglandin synthesis, necessary in gastric cytoprotection and platelet aggregation. Cox-2, whilst constitutively expressed in some tissues, including the vascular endothelium, is predominantly induced in response to inflammatory, injury and malignancy[61]. Most NSAIDs are non-selective; however celecoxib is an example of a selective inhibitor of cox-2, developed with the aim of avoiding the gastrointestinal toxicities, attributed to cox-1 inhibition.

Up-regulation of the cox-pathway is associated with the development or progression of cancers including colon, lung, breast, prostate, bladder and oesophageal[62]. For example, PCa is associated with a 3.4 fold increase in mean cox-2 levels relative to surrounding normal tissue, and overall cox-2 overexpression is detected in 83%[63]. Cox-2 overexpression results in increased prostaglandin synthesis, which itself is associated with more advanced disease[64]. Consistent with this, administration of prostaglandins to prostate cancer cells significantly increases cellular proliferation and increases cox-2 expression. The therapeutic relevance of this is supported by *in vivo* evidence that shows selective cox-2 inhibition suppresses tumour growth through induction of apoptosis and inhibition of angiogenesis through the down-regulation of endothelial growth factors[65].

Aspirin is a non-selective cox-inhibitor, also proposed as a re-purposed anti-cancer therapy. In contrast to other NSAIDs, aspirin is an irreversible cox-1 inhibitor, but due to its short half-life and extensive first pass metabolism, is unlikely to exert a systemic effect on the cox-pathway. Instead, aspirin exerts its predominant therapeutic effect on mature platelets, which contain cox-1 but as they lack a nucleus, are unable to resynthesize cox enzymes and so are vulnerable to irreversible cox inhibition[66]. Reduced platelet aggregation is one proposed mechanism through which aspirin may exert an anti-cancer activity, supported by the evidence demonstrating platelets facilitate metastatic spread[67]. However, distinct to cox-2 selective inhibition, aspirin is also proposed to modify the host environment through non cox-dependent pathways that include inhibition of angiogenesis,

promoting apoptosis and modification of pathways that promote cancer growth, such as nuclear factor-kappa B (NF-κB) and tumour necrosis factor (TNF) signalling[68].

Pre-clinical data

The therapeutic mechanism of NSAIDs in carcinogenesis is proposed to involve alteration of apoptosis, angiogenesis and immune function[68,69]. Pre-clinical evidence of anti-cancer activity has been demonstrated in multiple tumour types including colorectal (CRC), breast and non-small cell lung cancers (NSCLC) thus providing a rationale to review clinical data from a range of cancers when seeking to contextualise the STAMPEDE results[70-72,73].

In CRC, malignant transformation is associated with cox-2 overexpression, detectable in 40% of adenomatous colonic polyps and increasing to 80% of CRC[74]. Pre-clinical and clinical data acquired in familial adenomatous polyposis (FAP), an inherited cancer-predisposition syndrome, demonstrates NSAID treatment restores apoptotic mechanisms and blocks tumour formation. Whilst cox-2 inhibition in pre-clinical models of CRC ameliorates tumour stimulated angiogenesis and neovascularization, providing a further therapeutic mechanism of anti-cancer effect[69,74-77].

Pre-clinical evidence obtained in PCa models suggests several proposed mechanisms of anti-cancer effect. Cox-2 has been shown to promote cellular invasion and metastases via activation of matriptase, a matrix degrading protease. In turn, matriptase expression has been shown to correlate with poor PCa outcome[78]. In a model of CRPC characterised by cox-2 overexpression, cox-2 inhibition was shown to supress matripatase-induced cell migration by 80% and invasion by 95%[79]. Multiple lines of evidence support celecoxib exerting a pro-apoptotic effect, all suggest that this effect is in part dependent on cox-2 expression [80-83]. Mechanisms differ, but include via inhibition of *AKT* phosphorylation or via TNF signalling pathway[81,82].

Pre-clinical data suggests aspirin and NSAIDs may exert an anti-cancer effect via separate mechanisms, therefore should be considered separately and may be expected to benefit different tumour types and settings. Aspirin is proposed to exert an anti-metastatic effect via the inhibition of platelet function. Platelets may promote cancer development and metastatic spread through facilitating cancer cell adhesion and transmigration; enabling evasion of immunosurveillance by adhering and protecting circulating cancer cells from natural killer cells, and altering the tumour microenvironment via transforming growth factor beta 1 (TGF-B) and NF-κB pathways to promote metastatic invasion[84,85]. In addition,

it has been proposed that aspirin, via inhibition of cox-1 may indirectly suppress the induction of cox-2 in distant nucleated cells within the tumour cells or microenvironment[86]. This suggested inter-play between cox-1 and cox-2 is supported by the demonstration that inhibition of either results in tumorigenesis in rodent models and provides an explanation for the clinical data suggesting low-dose aspirin, though insufficient to exert systemic cox-inhibition, may have an anti-cancer effect[87]. For these reasons, when seeking supporting data for the celecoxib-ZA results, I will focus on data acquired with selective cox-2 inhibitors only.

Clinical data

Epidemiological data suggests NSAID use is associated with reduced PCa incidence, especially aspirin which is the most frequently studied association, see **Table 7.** In a systematic meta-analysis, data was pooled from 10 case-control and 14 cohort studies, including a total of 24,230 PCa cases in NSAID exposed populations. The results suggest NSAID use is associated with reduced PCa risk; Pooled Odds Ratio (POR) 0.89; 95% CI; 0.73-1.09; p=0.26. This trend was confirmed in non-aspirin NSAID exposed populations (POR 0.90; 95% CI 0.80-1.01, p=0.087) but only reached statistical significance in the largest population (17 studies) exposed to aspirin (POR 0.83; 95% CI 0.77-0.89, p<0.001)[88.] Included trials were heterogeneous; study design, outcomes and NSAID exposure all varied, limiting the ability to inform as to the impact of NSAID dose or duration.

Some of the strongest clinical evidence for an anti-cancer effect of NSAIDS is observed with aspirin, acquired through secondary meta-analyses of cancer incidence amongst participants in vascular prevention trials. In a meta-analysis of 17285 participants in 5 RCTs, a reduced incidence of metastatic cancer was observed in those randomised to aspirin (HR 0.64; 0.47-0.84, p<0.001) and a strong trend toward a reduction in PCa deaths, evident after 5-years follow-up (HR 0.52; 0.20-1.34)[89]. Consistent with this, retrospective review of aspirin use has shown an association with improved outcomes following radical treatment in three cohorts, with the greatest benefit observed in men with high-risk localised disease. In a review of 2051 men on surveillance following radical PCa treatment aspirin non-use was associated with earlier biochemical failure on multivariable analysis (aspirin non-use OR 2.05; 95%CI 1.33-3.17; p=0.0012)[90]. In a second cohort of 5955 men treated for localised PCa whilst also receiving anti-coagulation therapy, aspirin, as opposed to other anti-coagulants, was associated with a significantly lower risk of prostate cancer specific mortality (PCSM) after a median follow up of 70 months (3% vs. 8%; p=<0.1). Multivariable

analysis confirmed aspirin use was independently associated with a lower risk of PCSM (adjusted HR 0.43; 95% CI, 0.21- 0.87; P=.02) and was associated with a reduced risk of disease recurrence and the development of bone metastases. In the largest cohort study which included 8427 men, a significant reduction in PCSM was seen in those with high-risk features (≥T3 or Gleason score ≥8) PCSM (HR = 0.60; 95% CI, 0.37 to 0.97)[91]. Together these data support the need for randomised trials to evaluate aspirin as an adjuvant anti-cancer treatment.

Table 7: Epidemiological data assessing effect of NSAID and PCa incidence/outcome

Reference	n	Setting	Results
Mahmud 2010 *et al*[88]	n=24,230 (total)	All stages of PCa	• Aspirin use (17 studies) OR=0.83 (0.77-0.89; p<0.001) • Non-aspirin NSAID use (12 studies) OR=0.90 (95% CI 0.80-1.01; p=0.087)
Zaorsky *et al.* 2012[90]	n=2,051	M0 post radical RT	• Aspirin non-user associated with shorter biochemical failure free survival time OR 2.05 (95% CI 1.33-3.17)
CaPSURE study Choe *et al.* 2012[91]	n=5,995	M0 post radical treatment	• Aspirin use associated with improved prostate cancer-specific mortality HR 0.43 (95% CI 0.21-0.87)
Jacobs *et al.* 2014[92]	n=7,118	High risk M0	• Retrospective review of aspirin use suggested associated with improved PCa mortality HR 0.60 (95% CI 0.37-0.97)
Rothwell *et al*[89]	n=17,285	All stages of PCa	• Aspirin use associated with reduced risk of death due to PCa OR 0.34 (95% CI 0.12-0.99) p=0.049
Downer *et al*[93] Physicians Health Study	n=22,071	All stages of PCa	• Current and past regular aspirin use associated with lower risk of lethal PCa(HR 0.68; 95% CI 0.52-0.89) • Post cancer diagnosis aspirin use was associated with improved survival post diagnosis HR 0.72 95%; CI 0.61-0.90

The Add-Aspirin trial (NCT02804815) is a phase III placebo controlled RCT which will provide much needed randomised prospective data to address the question of whether adjuvant aspirin, given at a dose of 100mg or 300mg for at least 5 years, can improve rates of disease recurrence and survival. This trial will aim to recruit 2120 men with intermediate or high-risk localised PCa and is designed to detect an 8% improvement in biochemical disease free-survival. Recruitment is ongoing to this, and three parallel cohorts which include radically

treated colorectal, gastro-oesophageal and breast cancer, with combined analyses of survival also planned[94].

2.1.2 Impact of external safety data

The acquisition of clinical data evaluating cox-2 inhibitors as anti-cancer agents has been hampered by concerns regarding cardiovascular toxicity. Although cox-2 inhibitors were originally developed with the aim of achieving an improved toxicity profile, in 2002 rofecoxib was withdrawn in response to data demonstrating an increased risk of cardiovascular events[95]. This prompted numerous trials evaluating cox-2 inhibitors to close early due to poor accrual, see **Table 8** for three such examples in PCa.

Table 8: Cox-2 inhibitor trials in PCa that closed early in response to external safety data

Trial ID	Patient Group	Treatment	Outcomes	Sample size and trial status
NCT00136487	Biochemical relapse post radical treatment	Celecoxib or Placebo	PSA doubling time	**Design:** Placebo controlled phase II/III **Accrual:** 85 (target unknown) **Recruitment period:** Oct-2002- Sept 2006 **Status:** Terminated early in response to external safety data **Conclusion:** Primary objective not met but PSA velocity decreased [96]
NCT00073970	Biochemical relapse post radical treatment	Celecoxib	PSA response	**Design:** Single arm phase II **Accrual:** 37 (target 100) **Recruitment period:** Apr-2003 – Jan 2006 **Status:** Terminated early in response to external safety data **Note:** Results not published
NCT01220973	Biochemical relapse post radical treatment	Celecoxib + atorvastatin	PSA response	**Design:** Single arm phase II **Accrual:** 27 (target unknown) **Open:** Feb-2009 **Status:** Terminated early; results not published

Date summarised from www.clinicaltrials.gov

Evidence of increased cardiovascular risk

Evidence of increased cardiovascular risk was shown for rofecoxib in two chemoprevention trials. The Adenomatous Polyp Prevention on Vioxx Trial (APPROVe) randomised 2586 patients with a history of colorectal adenomas to receive 3 years of treatment with rofecoxib or placebo. The trial was halted due to an increase in thrombotic cardiovascular events, predominantly myocardial infarctions (MI) and ischaemic strokes, in the rofecoxib-treated group. Although the absolute risk was small, the corresponding relative risk (RR) is clinically significant when considering the setting: the evaluation of a preventative treatment in an otherwise healthy population. 46 confirmed thrombotic events occurred in the treatment group compared with 22 in the placebo group, corresponding RR 1.92; 1.19-3.11; (p=0.008); which, when adjusted for time on follow-up, represents a difference of 1.50 events vs 0.78 events per 100 patients years[95]. This prompted a review of accumulating safety data in a second trial, Adenoma Prevention with Celecoxib in which 2035 patients were randomised 1:1:1 to receive placebo, celecoxib 200mg BD or celecoxib 400mg BD. Blinded review of all deaths and non-fatal cardiovascular adverse events demonstrated a dose-related increase in cardiovascular-related mortality for celecoxib. The annual incidence of death from cardiovascular causes was 3.4 per 1000 in the placebo group, increasing to 7.8 per 1000 and 11.4 per 1000 in the 200mg and 400mg celecoxib treatment groups respectively[97].

These data fuelled the concern that this was a class effect of selective cox-2 inhibitors, hypothesised to be due to an imbalance between prothrombotic and anti-thrombotic eicosanoids resulting from the lack of cox-1 inhibition. Selective cox-2 inhibition reduces the production of prostacyclin, but without affecting the Cox-1 dependent synthesis of thromboxane A2, a promoter of platelet aggregation and vasoconstriction. Cox-2 expression is increased in the presence of endothelial injury and therefore suppression of cox-2 dependent prostaglandin I_2 and the resulting pro-thrombotic imbalance may be most clinically apparent in those with the highest intrinsic risk of atheromatous cardiovascular disease[98]. The latter providing a rationale for the observation that greatest increased relative risk was seen in patients with a history of cardiovascular disease[99,100]. Although the clinical data for increased cardiovascular risk for cox-2 inhibitors other than rofecoxib was inconsistent, significant concern remained and many trials closed early[101,102].

In 2017, a meta-analysis has shown that the assumption that cox-2 selectivity confers increased cardiovascular risk cannot be supported, instead demonstrating this to be specific

to rofecoxib[103]. In total, 26 RCTs or prospective cohort studies evaluating NSAIDs in a range of disease settings were identified where cardiovascular outcome data was also included. This represents a total of 228,391 patients, of which 65,341 were exposed to celecoxib. Comparisons were made between cox-2 inhibitors, non-selective NSAIDs or placebo groups, depending on the trial design. The meta-analysis used the primary outcome of MI, stroke or cardiovascular death, or any combination of these. The findings demonstrate that the adverse cardiovascular events may not be based on the cox-2 selectivity of NSAIDs, as rofecoxib appears to be the only NSAID to confer increased cardiovascular risk. When compared to placebo, rofecoxib was associated with an increase in cardiovascular events: OR 1.572; 95% CI 1.123-2.201; p=0.008. When rofecoxib is removed from the cox-2 inhibitor group no difference in cardiovascular outcome is observed, consistent with this single agent skewing the data. The results of this meta-analysis are reassuring when considering the clinical relevance of the STAMPEDE celecoxib-ZA results. Yet the impact the toxicity data had at the time cannot be reversed, influencing the perceived role of celecoxib in this setting and delaying data acquisition.

2.1.3 Rationale for evaluating in combination with zoledronic acid

ZA is a bisphosphonate shown to have anti-cancer effects in models selected due to the predominance of metastatic bone involvement e.g. breast, prostate and lung cancer. Bisphosphonates bind preferentially to bone where they are ingested by osteoclasts leading to inhibition of bone reabsorption and osteoclast apoptosis[104]. The strength of evidence of anti-cancer effect is greatest for the second generation nitrogen-containing compounds, such as ZA. Studies using PCa cells lines demonstrate ZA inhibits prostate tumour cell adhesion, migration and invasion suggesting it may act to prevent metastatic spread[105-107]. In addition to direct anti-tumour cell effects, ZA is proposed to have an immune modulatory function via stimulating γδ T cells[108]. This T cell subpopulation is capable of exerting an anti-tumour response through the recognition of phosphoantigens overexpressed in some cancer cells. ZA treatment has been shown to stimulate γδ T cell expansion and function in PCa patients[108]. However, this is not PCa specific as breast cancer cells are also more vulnerable to γδ T cell mediated cell death following ZA treatment[108,109]. Therefore this represents a further proposed mechanism by which ZA may control cancer cell progression and may be relevant when considering the observed additive effect observed when given in combination with celecoxib.

ZA was evaluated alone and in combination with celecoxib within STAMPEDE, hypothesised to improve FFS and OS. In 2002, ZA was licenced for use in mCRPC having been shown to prolong the time to first skeletal-related event (SRE), a composite outcome measure used to describe the major complications of metastatic bone involvement: pathological fracture, spinal cord compression, or pain or impending fracture, necessitating radiotherapy or surgery[110]. SREs are a major cause of significant health and economic burden and can lead to severe pain, reduced quality of life, disability and increased risk of death[111]. The use of ZA was shown to reduce the risk of an SRE (RR 0.64 (95% CI 0.49-0.85)[112]. Prior to STAMPEDE opening, the MRC PR05 trial reported a survival improvement with sodium clodronate in mCSPC and a second trial (PR04) was underway in M0 disease. Intriguingly, updated survival data from PR05 reported in 2009 also suggest a selective beneficial effect metastatic castrate-sensitive disease, (HR 0.77; 95% CI 0.60-0.98; p=0.032)[113]. No evidence of benefit was ultimately seen in the M0 population in PR04 (HR 1.12; CI 0.89-1.42; p=0.94). However despite evidence of benefit with this first generation bisphosphonate, these data did not impact practice and cannot be supported by the results of the STAMPEDE ZA comparison (HR 0·94; 95% CI 0·79–1·11; p=0·450). A STOpCaP meta-analysis identified seven RCTs evaluating bisphosphonates in mCSPC and showed that when PR05 was excluded there was no evidence of benefit and specifically, there was no evidence that ZA improved OS (HR 0.94; 0.83-1.07; p=0.323)[22].

Bisphosphonate use

Current guidance recommends ZA is considered for men with metastatic prostate cancer at high risk of SREs, although NICE guidance states use should be restricted to symptomatic groups in whom other treatments, including analgesics and palliative radiotherapy have failed[114,115]. The recent demonstration that a 12-weekly schedule is non-inferior to 4-weekly is expected to support the cost-effectiveness argument and mean that this continues to be a useful adjunct in the management of metastatic disease[116,117]. Guidance for use in other tumour sites varies, reflecting the varying strength of evidence of benefit[110]. When seeking to contextualise the celecoxib-ZA results, studies evaluating cox-2 inhibitors as treatments for metastatic breast, prostate or NSCLC were considered suitable for inclusion. The rationale being that bisphosphonates were introduced in these indications during the time period in which cox-2 inhibitors were under evaluation within clinical trials.

Bisphosphonates were recommended for use in patients with metastatic breast cancer as early as 1999[118]. This was based on accumulating evidence published between 1993 and 1998 demonstrating they were beneficial adjuncts to chemotherapy, prolonging the time to first SRE and reducing the number overall[119]. In 2000, the American Association of Clinical Oncology (ASCO) guidance recommended bisphosphonates may be used in addition to SOC for women symptomatic from metastatic bone disease based on the evidence they reduced SREs[120].

The introduction of bisphosphonates as part of the SOC treatment for NSCLC was later. In 2003, ZA was shown to reduce the risk of SREs by 31% (HR 0.693; p=0.003) in a placebo-controlled RCT of 773 patients, including 224 with NSCLC[121]. This led to both the American and European Society of Medical Oncology (ESMO) guidance supporting use from 2008 and 2009 respectively[122,123]. It is therefore anticipated that a proportion of NSCLC trials identified may have permitted bisphosphonate use as part of protocol therapy so be able to provide data relevant to contextualising the celecoxib-ZA combination.

In summary, the evaluation of both celecoxib and ZA was supported by pre-clinical, epidemiological and clinical data. STAMPEDE aimed to assess the impact on OS of adding both, alone or in combination, in the setting of high-risk M0 or mCSPC . The rationale for the combination was that the differing proposed mechanisms of action may be complementary and deserved evaluation as a novel combined approach. Results show that the treatment combination was well tolerated with no excess in cardiovascular toxicity when celecoxib was given for the planned maximum duration of 1 year. This is further supported by the recently published meta-analysis which did not find evidence of increased cardiovascular risk associated with celecoxib[103]. Within the metastatic subgroup, the estimated treatment effect of celecoxib-ZA is clinically and statistically significant (HR 0.78; 95%CI 0.62-0.98) and comparable with that shown for the addition of docetaxel (HR 0.76; 95%CI 0.62-0.92), which has changed practice[20,23,24]. As a well-tolerated generic drug combination, this therapeutic strategy could be provided in both middle and high income countries and therefore has the potential to impact on global PCa outcomes. However importantly, in contrast to docetaxel, there is no known external clinical data supporting the activity of this combination. Additionally, the biological rationale for the observed selective and additive effect is poorly understood.

2.1.4 Research aims

In this chapter I will aim to address the following research question and objectives:

Research question

- Can the STAMPEDE result demonstrating a clinically significant improvement in survival with the combination of celecoxib-ZA be supported by external clinical data?

Objectives

- To undertake a systematic review and meta-analysis of relevant clinical data
- To explore pre-clinical data to generate hypotheses as to the therapeutic mechanism of action that is selective to metastatic disease

2.2 Methods

Methods for this systematic review and meta-analysis were described in a prospectively registered protocol available online (PROSPERO registration: CRD42016041743), available via www.crd.york.ac.uk/prospero and in **Appendix D**. This review was conducted and reported in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines[124].

2.2.1 Search strategy

Electronic searches of databases (Medline, Embase, and Cochrane Central Register of Controlled Trials), clinical trial registries (clinicaltrials.gov) and conference proceedings (ASCO and ESMO) were undertaken. All sources were searched from 1996 (when selective cox-2 inhibitors were first clinically evaluated) until 15-May-2017, except conference proceedings which were limited to 2004, when electronic records exist. For details refer to protocol in **Appendix D**. Bibliographies of full reports and review articles were searched in order to identify further potential eligible studies. All trials are summarised in **Table 9**.

2.2.2 Outcomes

The primary outcome was PFS defined as the time from randomisation to the first evidence of symptomatic or radiological progression or death. The secondary outcomes were OS,

defined as the time from randomisation to death from any cause; disease-free survival (DFS), defined as the length of time following completion of treatment during which there are no signs or symptoms of cancer recurrence and overall response rate (ORR), defined as the proportion of patients with documented partial (PR) or complete radiological (CR) response according to RECIST criteria[125]. Further secondary outcomes were cardiovascular outcomes, where available. Translational outcomes were also recorded to support discussion of potential therapeutic mechanisms of action.

2.2.3 Study selection

All identified studies were assessed for eligibility and where insufficient outcome data was available, additional data was requested from the authors. Where multiple publications had arisen from a single study the most recent was selected. Articles were grouped according to cancer histology and setting e.g. neo-adjuvant, adjuvant or metastatic.

2.2.4 Eligibility criteria

Phase II and III RCTs were eligible if evaluating selective cox-2 inhibitors as cancer therapies. All trials were required to be controlled with the comparator group receiving SOC or placebo. Cox-2 inhibitors could be given alone or in combination with additional research treatments, or the current SOC. Searches were conducted with no restriction on language however data extraction relied on sufficient information in English. All identified studies evaluating cox-2 inhibitors as treatments for metastatic cancer, containing sufficient outcome data for PFS, OS or RR were eligible for inclusion in the meta-analysis. Exclusion criteria included trials evaluating unselective cox-2 inhibitors e.g. aspirin; primary chemopreventive trials, or trials with active control arms e.g. cox-2 inhibitor vs. cox-2 inhibitor + experimental agent.

Modified eligibility criteria

As initial searches failed to identify any RCTs evaluating the combination of a cox-2 inhibitor and bisphosphonates, the eligibility criteria were broadened by removing the bisphosphonate search terms to include RCT evaluating cox-2 inhibitors. In order to seek relevant data to contextualise the results of the STAMEPDE, meta-analyses were limited to metastatic solid organ cancers, with the primary focus of interest being cancers where bone metastases are common and bisphosphonates may have been given; prostate, NSCLC and breast cancer.

2.2.5 Data items and collection

The following data items were extracted using a standardised pre-designed format from all eligible studies:

1. *Trial design* - including phase, number of treatment arms, comparator, outcomes
2. *Trial conduct* - including accrual target and period, participating countries and where trials terminated early, the reason for discontinuation.
3. *Participants' details* - including cancer type and treatment setting
4. *Intervention* - specific cox-2 inhibitor, dose and duration
5. *Outcomes* – included PFS, OS, RR, cardiovascular and translational outcomes

Methodological quality was assessed using the Cochrane Collaboration tool for assessing the risk of bias[126]. Information was extracted on the method of randomisation sequence generation, allocation concealment, blinding, completeness of outcome data and whether all outcomes were reported or available; results of this assessment are summarised in **Figure 10**- **Figure 12**.

Additional data collection of bisphosphonate use

Protocols for trials in metastatic NSCLC and breast cancer were searched for electronically, and requested from all corresponding authors to determine if bisphosphonates were allowed as protocol permitted SOC. Relevant clinical guidance was also reviewed.

2.2.6 Statistical analysis

I undertook all statistical analyses supported by the meta-analysis group at MRC CTU at UCL. HR and associated statistics (95% confidence interval, p value and sample size) were extracted from all study reports. Where unavailable, authors were contacted and if necessary, estimations were made from the Kaplan-Meier curves or other available summary statistics using published methods[127-129].

Where sufficient data were available PFS, OS or RR a meta-analysis was conducted in which HRs were combined across the trials using a fixed-effect model (Mantel-Haenzsel). The appropriateness of this model, which assumes no statistical heterogeneity, was assessed using the I^2 statistic and χ^2 test. In the case of significant heterogeneity, a random-effect model was used to assess the robustness of the results to the choice of model[130]. Probability values were two-sided, with $p<0.05$ considered statistically significant. Analyses were performed in Review Manager Version 5.3.

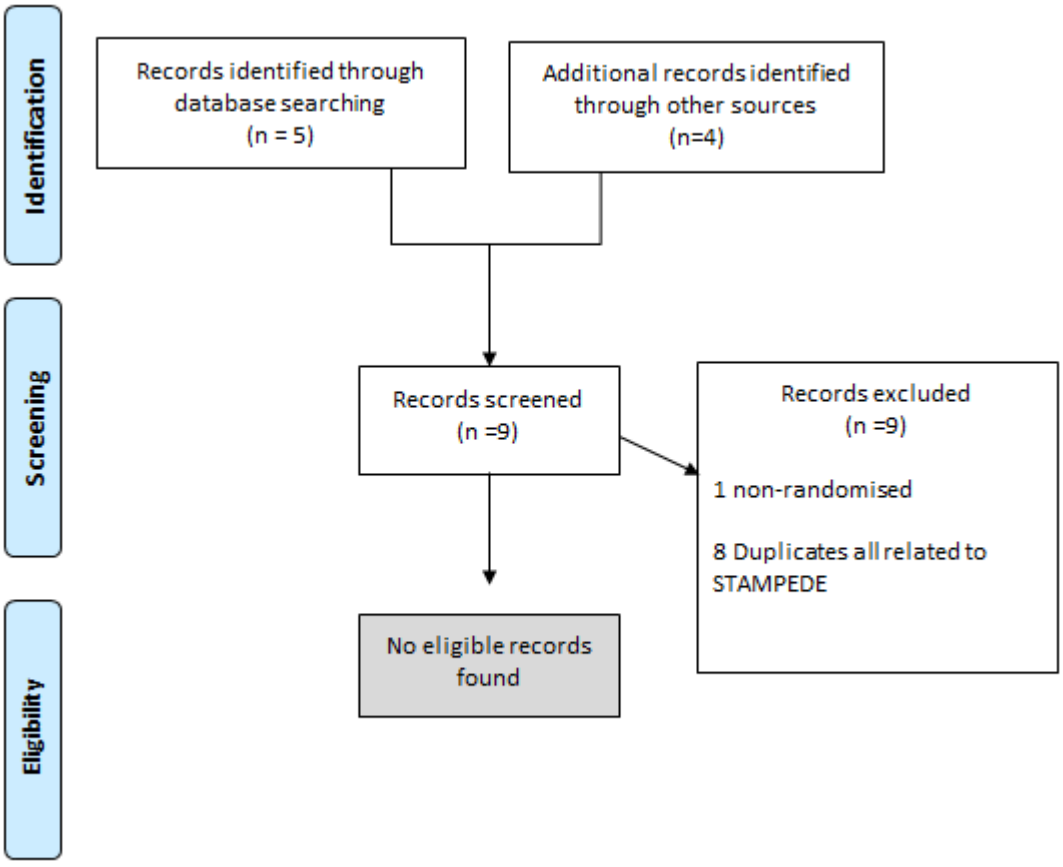
2.3 Results

2.3.1 Study selection

Initial searches failed to identify any other studies using the original search criteria that combined both cox-2 search terms and bisphosphonate search terms. All eligible studies were references to the STAMPEDE trial. There was no randomised data evaluating the combination in any other cancer setting, see

Figure 8.

Figure 8: PRISMA diagram



Using the modified inclusion criteria 555 study reports were screened and 50 eligible studies identified, see **Figure 9**. Reasons for exclusion included non-randomised design, pre-clinical studies and trials evaluating cox-2 inhibitors as chemopreventive agents or for symptom control. One study was excluded as it included an active control arm, another which enrolled patients with different types of cancer was excluded as it was not possible

to extract sufficient data for each type individually. 16/50 studies contained sufficient data for PFS, RR and/or OS.

Figure 9: PRISMA diagram using modified search criteria

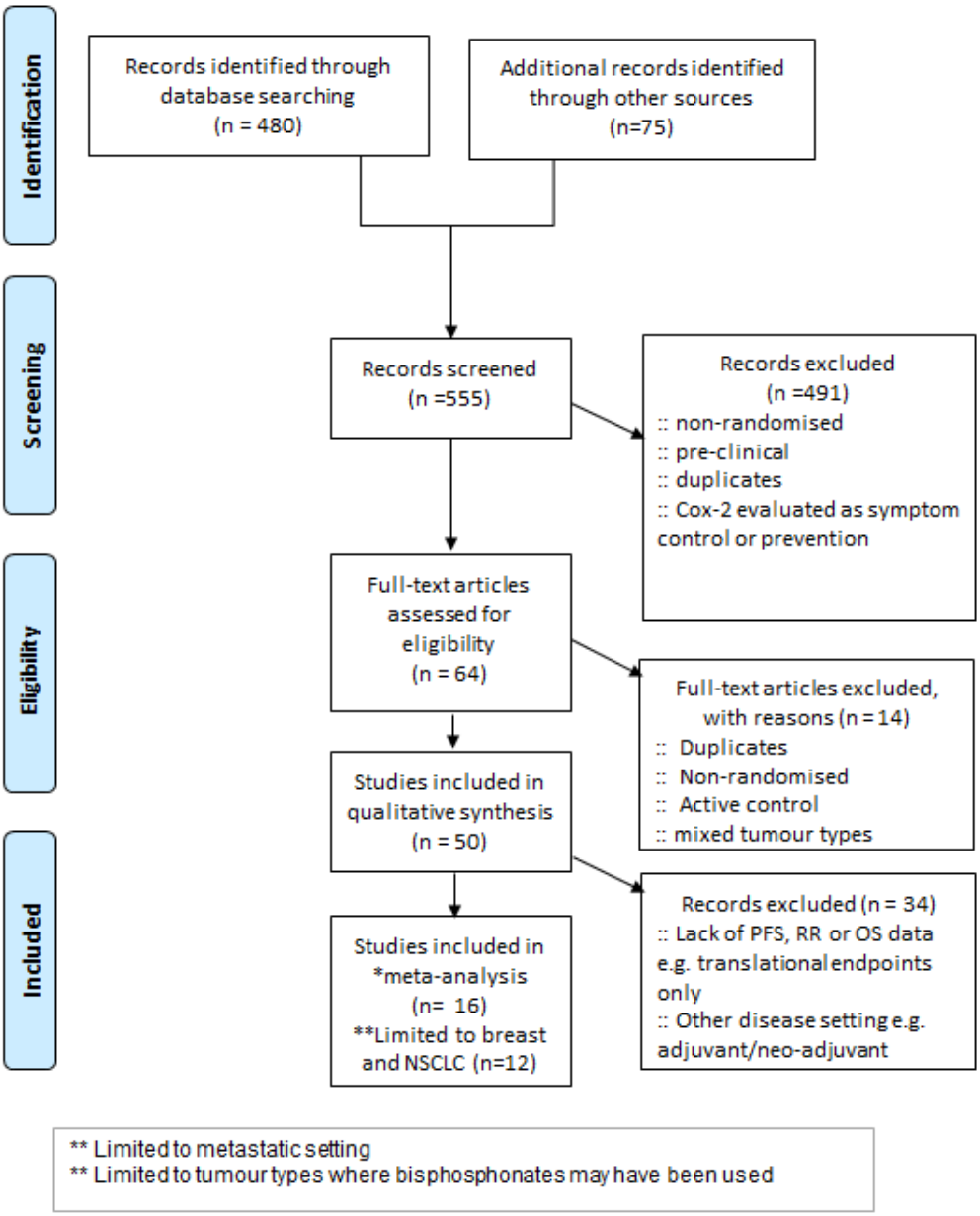


Table 9: Summary of trials identified

Site	Total no trials	Split by disease setting			Split by outcome			Translational outcome only	Total no of trials in M1 setting with efficacy data	Notes
		Neo-adjuvant	Adjuvant	Metastatic	PFS	OS	RR			
NSCLC	14	0	0	14	9	8	6	1	10	5 authors contacted Unpublished data provided for one study
Breast	12	7	4	2	2	1	2	2	2	3 authors contacted, no response
Prostate	4	2	1	1	1	1	0	2	1	All trials excluding STAMPEDE designed with PSA or pathological endpoints
Colorectal	9	2	2	5	2	3	5	1	4	3 authors contacted Meta-analysis includes published and unpublished data available on clinicaltrials.gov
HBP	2	0	0	2	0	0	0	0	0	Both trials in hepatocellular carcinoma
Gastro-oesophageal	4	3	0	1	1	0	1	0	0	Only one article available in English
Urothelial	3	2	1	0	0	0	0	1	0	
Gynae	2	0	0	2	1	1	0	0	1	

Key: NSCLC Non-small cell lung cancer, HBP Hepato-Pancreato-Biliary, Gynae Gynaecological, PFS progression free survival, OS overall survival, RR response rate, tumour types of interest highlighted by red shading

2.3.2 Study characteristics

No trials evaluating cox-2 inhibitors in combination with bisphosphonates were identified. Therefore all trials identified using the modified search criteria (n=50) were evaluating cox-2 inhibitors given alone, or in combination with SOC. The majority of eligible studies recruited patients with NSCLC (n=14), breast cancer (n=12) or CRC (n=9). STAMPEDE was the only identified trial in metastatic prostate cancer. Other tumour types included gastro-oesophageal (n=4), hepatocellular carcinoma (n=2), urothelial (n=3) and gynaecological (n=2); see **Table 9**.

Trial conduct

All eligible trials were controlled; the comparator group receiving placebo (n = 25) or SOC, which included observation in adjuvant trials. All trials were conducted between 2000 and 2016, with the majority of trials recruiting between 2002 and 2011. 13/50 trials closed early or failed to reach the target accrual including two trials which discontinued due to early concerns about toxicity.

A neo-adjuvant study prior to radical prostatectomy reported by Flamiatos *et al* closed to recruitment following a post-operative MI occurring in a patient receiving celecoxib, see **A1: Table 67**: Summary of ineligible PCa trials[131]. Additionally, a factorial study in CRC reported by Kohne *et al* which compared capecitabine and irinotecan (CAPIRI) and 5-fluorouracil/folinic acid/irinotecan (FOLFIRI), with a second randomisation between celecoxib or placebo, also closed early due to excess toxicity associated with CAPIRI. Increased neutropenic infection, thromboembolic events (predominantly pulmonary emboli) and diarrhoea were observed when CAPIRI was combined with placebo or celecoxib, see **Table 13**[132].

External safety data was cited in four trials that discontinued early, see **Table 8** and **Table 10**[96,133-135]. One trial in NSCLC closed due to poor accrual[136] and the evaluation of rofecoxib in NSCLC was terminated following its withdrawal, see **Table 10**[137].

Disease setting

In NSCLC all trials were in the metastatic setting, see **Table 10** and **Table 11**. In breast cancer, the majority of trials were in the neo-adjuvant setting with cox-2 inhibitors given prior to surgery and assessed using pathological response rate (pRR). Four were adjuvant trials assessing improvements in DFS, see **Table 64** and **Table 65** in **Appendix A1**. Only two breast cancer trials were in the metastatic setting of interest; see **Table 12.** Out of 9 CRC

trials, five were in metastatic disease, however as bone metastases are infrequent, this was not defined as a tumour site of interest, see **Table 13.** The remaining 4 CRC trials were split between neo-adjuvant and adjuvant settings; see Table 66 in Appendix A1. 3 out of 4 studies in gastro-oesophageal cancer were in the neo-adjuvant setting, one enrolled patients with metastatic disease and only one study in gynaecological cancer included patients with metastatic disease.

Treatment exposure

The most common cox-2 treatment was celecoxib (44/50 studies); rofecoxib was given in 4 studies, whilst two evaluated etorcoxib or apricoxib. Treatment exposure varied by tumour site, disease setting and was shortened due to premature trial closure in 6 studies[96,133-135,137]. The most frequent per-protocol dose of celecoxib was 400mg BD.

Metastatic CRC: In all trials cox-2 inhibitor treatment was planned to be given until progression however 2/4 trials terminated early limiting treatment exposure, see **Table 13.**

Metastatic NSCLC: In 4/14 trials the maximum duration of cox-2 treatment was capped at between 1 to 3 years. In the GECO trial, rofecoxib was evaluated in metastatic NSCLC however exposure was curtailed when the drug withdrawn[137]. Of the 118 patient treated with rofecoxib, the median treatment duration was 14 weeks (0-64), 33 patients received treatment for more than 6 months and only one received >1 year[137].

Breast cancer: The majority of trials were in the neo-adjuvant setting or adjuvant setting and the typical treatment duration was 3-6 months or 2 years respectively. In the largest study in advanced breast cancer the treatment exposure was limited following trial termination after 127 of the planned 342 were enrolled. At this point 85 patients were still receiving treatment, all of whom were stopped, limiting the median duration of treatment to 5.8 months[133]. In PCa the planned neo-adjuvant treatment duration was between 4-6 weeks and the maximum treatment duration in the adjuvant or metastatic setting was capped at 1 year, a change prompted by external safety data.

Outcomes

In total, sufficient PFS data was obtained for 16 studies. When limiting to the trials in the metastatic tumour sites of interest, this includes 9 studies in NSCLC (1894 participants) and 2 studies in breast cancer (257 participants). OS data were available for 14 studies; 8 studies in metastatic NSCLC (1824 participants). In total, ORR data was available in 6 studies, including 7 in metastatic NSCLC (1512 participants) and 2 in metastatic breast

cancer (234 participants). Although of less relevance due to the lack of frequent bone metastases, sufficient data for PFS, OS and ORR was obtainable for 298, 515 and 683 participants respectively with metastatic CRC through the meta-analyses of 4 studies.

Cardiovascular data was only reported in two trials. The only trial to suggest increased toxicity was reported by Groen *et al.* which randomised 561 patients with metastatic NSCLC to SOC chemotherapy with celecoxib or placebo. A greater absolute number of cardiovascular events were observed in celecoxib-treated patients; events included pulmonary embolism, pericardial effusion, atrial fibrillation, hypertension and an arterial thrombus. It should be noted that numbers were small (6 versus 3) and this difference was not statistically significant (p=0.50)[138]. A second trial closed early due to increased toxicity reported by Fuchs *et al* attributed to CAPIRI chemotherapy, which was being compared with FOLFIRI, with celecoxib or placebo in a factorial design. 7 treatment-exacerbated deaths occurred, however importantly this excess toxicity was attributed to the chemotherapy regime (CAPIRI). 4 fatal events occurred in patients receiving CAPIRI+ placebo, and 2 in CAPIRI + celecoxib, with 2 in FOLFIRI and celecoxib. The reported treatment related toxicities were gastrointestinal, neutropenic infections or thromboembolic events. These resulted in recruitment being halted after 85 of the planned 629 patients were accrued[132].

Table 10: Summary of eligible NSCLC trials -1

Publication	Setting	Article type	Accrual			Trial design		Treatment	Outcomes				Trial status
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration	PFS	OS	RR	Non-published data	
Lilenbaum *et al.* 2006[136]	Metastatic 2nd line	Full	USA	2002 to 2003	133 (432)	Phase II Factorial	SOC chemo vs. non-standard chemo +/- cox-2 inhibitor	Celecoxib 400mg BD until progression	✓	✓	NR		Discontinued due to poor accrual
De Ruysscher *et al.*2007[139]	Locally advanced/ metastatic	Full	Holland	2003 to 2004	42 (102)	Phase II	SOC RT + cox-2 inhibitor SOC RT + placebo	Celecoxib 400mg BD until progression or max 2yrs	✓	✓	✓	Yes	Did not reach target accrual
Gridelli *et al.* 2007 GECO[137]	Metastatic 1st line	Full	Italy	2003 to 2004	400 (229)	Phase III Factorial	SOC chemo vs. non-standard chemo +/- cox-2 inhibitor	Rofecoxib 50mg OD until progression	✓	✓	✓		Cox-2 comparisons terminated early
Zhou *et al.* 2007[140]	Metastatic 1st line	Abs	China	NK	65 (NK)	Phase II	SOC chemo +cox-2 inhibitor SOC chemo	Celecoxib 400mg BD	NR	NR	NR		Completed Insufficient data
Koch *et al.* 2011 CYCLUS[141]	Metastatic 1st line	Full	Sweden	2003 to 2006	316 (760)	Phase III	SOC chemo +cox-2 inhibitor SOC chemo +placebo	Celecoxib 400mg BD until progression or max 1yrs	✓	✓	✓		Completed, failed to reach target accrual
Groen *et al.* 2011 NVALT-4[138]	Metastatic 1st line	Full	Holland	2003 to 2007	561 (NK)	Phase III	SOC chemo + cox-2 inhibitor SOC chemo +placebo	Celecoxib 400mg BD until progression or max 3yrs	✓	✓	✓		Completed

Key: CYCLUS, CY-cyclooxygenase-2 inhibitor; Chemotherapy, LUng cancer, Survival; GECO, GEmcitabine-COxib in NSCLC; NVALT, Nederlandse Vereniging van Artsen voor Longziekten en Tuberculose; NR, Not reported; NK, Not known.

Table 11: Summary of eligible NSCLC trials -2

Publication	Setting	Article type	Accrual			Trial design		Treatment	Outcomes				Trial status
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration	PFS	OS	RR	Non-published data	
Gitlitz *et al.* 2014[142]	Metastatic 1st line	Full	USA	NK	120 (NK)	Phase II	Erlotinib + cox-2 inhibitor Erlotinib + placebo	Apricoxib 400mg OD	✓	✓	✓		Completed
Reckamp *et al.* 2015[143]	Metastatic 2nd line	Full	USA	2007 to 2011	107 (NK)	Phase II	Erlotinib + cox-2 inhibitor Erlotinib + placebo	Celecoxib 600mg BD given until progression or max 1year	✓	✓	✓		Completed
Edelman *et al.* 2015[144]	Metastatic 2nd line	Full	USA	UK	72 (NK)	Phase II	SOC Chemo + cox-2 inhibitor SOC Chemo	Apricoxib 400mg OD	✓	NR	NR		Completed
Edelman *et al.*2017[145]	Metastatic 1st line	Full	USA	2010-2013	312 (322)	Phase II	SOC Chemo + cox-2 inhibitor SOC Chemo + placebo	Celecoxib 400mg BD until progression	✓	✓	NR	Pre-publication data provided	Completed

Key: NR, Not reported

Table 12: Summary of eligible metastatic breast cancer trials

Publication	Setting	Article type	Accrual			Trial design		Treatment	Outcomes				Trial status
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration	PFS/TTP	OS	RR	Contacted	
Dirix *et al.* 2008[146]	Metastatic	Full	Multi-national	2002	111 (100)	Phase II	Exemestane Exemestane + cox-2 inhibitor	Celecoxib 400mg BD	✓	NR	✓	Yes: no additional data	Completed
Falandry *et al.*2009 GINECO study	Metastatic	Full	France	2003 to 2004	157 (342)	Phase III	Aromatase inhibitor + placebo Aromatase inhibitor + cox-2 inhibitor	Celecoxib 400mg BD	✓	NR	✓	Yes: no response	Terminated early

Key: NR, Not reported

Table 13: Summary of trials in metastatic colorectal cancer

Publication	Setting	Article type	Accrual			Trial design		Treatment	Outcomes				Trial status
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration	PFS/TTP	OS	RR	Contacted	
Jin *et al.*2011[147]	Metastatic	Full	China	2005 to 2008	90	Phase II	SOC Chemo + cox-2 SOC Chemo	Celecoxib 400mg for min 8 weeks	NR	NR	✓		
Fuchs *et al.*2007[134]	Metastatic 1st line	Registry results summary only	North America & Australasia	2003 to 2004	430	Factorial phase III	*1st randomisation* Chemo 1 Chemo 2 Chemo 3 *2nd randomisation* cox-2 placebo	Celecoxib 400mg BD	✓	✓	✓	Yes: no response	Terminated early due to external safety concerns
Kohne *et al.*2008[132]	Metastatic	Full	EORTC group	2003 to 2004	85 (6290)	Factorial phase III	*1st randomisation* Chemo 1 Chemo 2 *2nd randomisation* cox-2 placebo	Celecoxib 400mg BD	✓	✓	✓	Yes: no response	Terminated early
Maiello *et al.*2006 GOIM[148]	Locally advanced or metastatic	Full	Italy	2003 to 2004	81	Phase II	SOC chemo + cox-2 SOC chemo	Celecoxib 400mg BD	NR	NR	✓	Yes: no response	Completed

Key: TTP, Time to progression; PFS, progression free survival; OS, overall survival; RR, response rate; NR, Not reported.

2.3.3 Risk of bias

The risk of bias within studies was evaluated using the Cochrane assessment tool and the consensus assessment is summarised in **Figure 10-Figure 12.**

NSCLC

The overall assessment of the nine NSCLC study reports was that there was a low risk of bias; however three potential sources were identified, judged likely to affect a total of four trials. The two unblinded trials were vulnerable to detection bias in outcomes of PFS and ORR, both relying upon investigator assessment with neither study reporting centralised or blinded review[136,137]. The CYCLUS (CY-cyclooxygenase-2 inhibitor, Chemotherapy, LUng cancer) reported by Koch *et al* excluded three ineligible patients from the efficacy analysis however the impact of potential attrition was judged to be small in a randomised population of 312 patients[141]. One trial were found to be at risk of selection bias due to inadequate detail of sequence generation and allocation concealment and baseline characteristics appear slightly imbalanced, reported by De Ruysscher *et al.* [139].

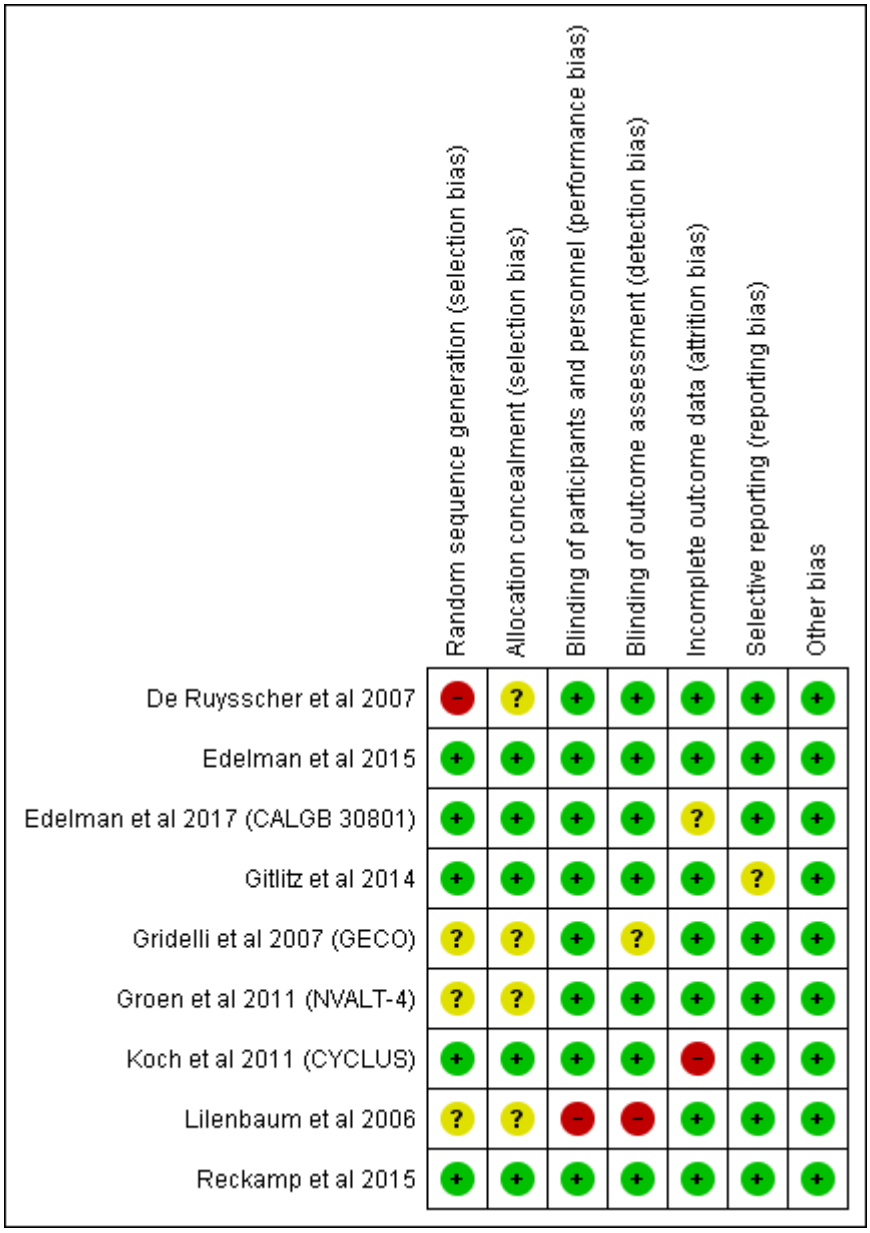
Breast cancer

Both studies are judged to be at potential risk of attrition and detection bias. Although balanced by group, Direx *et al* .report only 100/111 (90%) of randomised patients were assessable for RR and TTP, exclusion reasons included failure to start treatment and treatment duration of <4 weeks[146]. In addition, the lack of blinding risks performance and detection bias in a trial where the primary endpoint was rate of clinical benefit based on radiological assessment, which although based on RECIST, was not otherwise blinded to treatment allocation. Despite terminating early, the second trial reported by Falandry *et al.* was judged to have less risk of bias, apart from potential attrition bias for ORR as only 85% were assessable for this outcome[133].

Colorectal cancer

All four studies are judged to be at some risk of bias; 3 studies are open label and therefore at risk of performance and detection bias of RR which was based on unblinded investigator assessment[132,147,148]. One study report was limited to information available on clinicaltrials.gov which lacks detail on randomisation and baseline characteristics meaning the risk of selection bias is unclear. In the same study, the higher number of withdrawals on the placebo arm and lack of detail on blinding means the risk of potential performance bias is also uncertain[134].

Figure 10: Cochrane risk of bias summary (NSCLC trials)



Key

Figure 11: Cochrane risk of bias summary (Breast cancer trials)

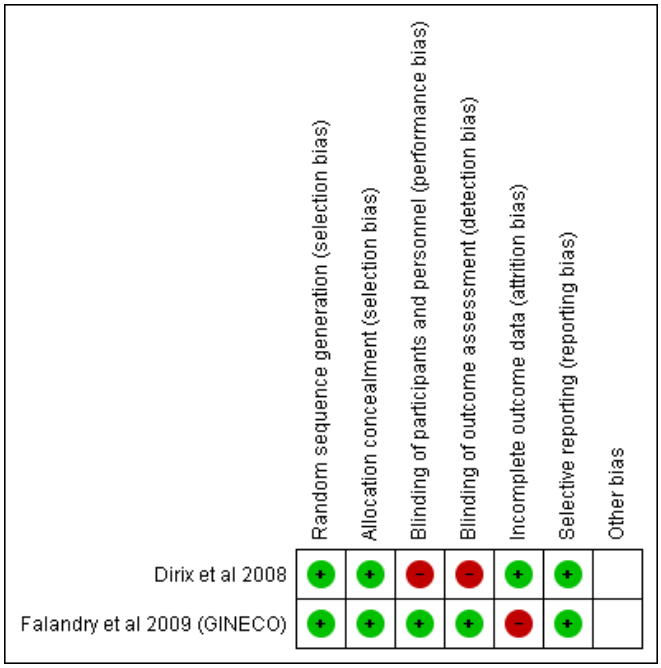
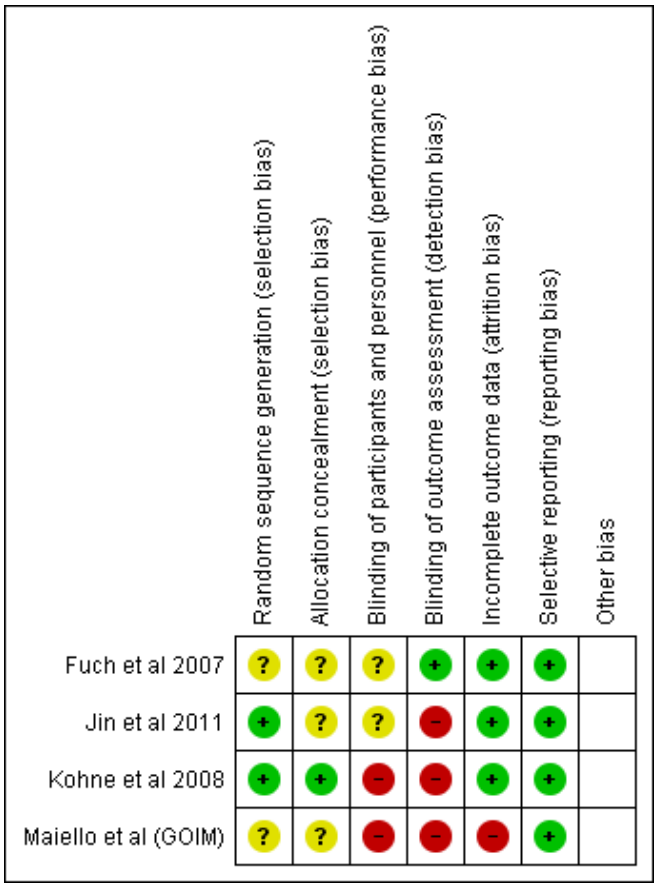
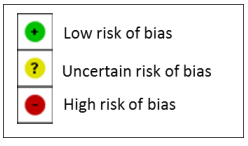


Figure 12: Cochrane risk of bias summary (CRC trials)



Key



2.3.4 Additional data on bisphosphonate use

Additional data regarding bisphosphonate use was sought for all studies in metastatic NSCLC and breast cancer. It was possible to contact 10 out of 11 authors and 6 responded see **Table 14**- **Table 16.** Overall, bisphosphonate use is judged unlikely in 3 trials, possible in 5 trials (all of which recruited in the USA, reflecting local guidance confirmed by two authors) and unknown in 2 trials. Two authors confirmed Dutch practice at the time would not have included bisphosphonates. Few trials collected data on concomitant medications; however the GECO trial team confirmed bisphosphonates were not listed for any of their participants. Neither author was able to provide additional details for the two breast cancer trials. Where it was recommended, clinical guidance suggested bisphosphonates were considered for symptomatic patients only; therefore overall it appears bisphosphonate use was uncommon.

Table 14: Bisphosphonate use in NSCLC trials -1

Study	Setting	Country	Accrual period	Contacted	Responded	Response details	Relevant Clinical Guidance	Bisphosphate Co-administration
Lilenbaum *et al.* 2006[136]	Metastatic 2nd line	USA	2002 to 2003	✓	✓	Bisphosphonates may have been used according to clinical guidance at the time; concurrent use not collected by the trial	Recommended in ASCO guidance since 2003	Possible
De Ruysscher *et al.*2007[139]	Locally advanced/ metastatic Stage II-III	Holland	2003 to 2004	✓	✓	Confirmed bisphosphonates not used as SOC at the time		Not used
Gridelli *et al.* 2007 GECO[137]	Metastatic 1st line	Italy	2003 to 2004	✓	✓	Whilst permitted by the protocol, trial statistician confirmed not recorded as a supportive medication for any trial participants therefore unlikely	Recommended in ESMO guidance since 2009	Unlikely
Zhou *et al.* 2007[140]	Metastatic 1st line	China	NK	X	n/a	n/a	NK	Unknown
Koch *et al.* 2011 CYCLUS[141]	Metastatic 1st line	Sweden	2003 to 2006	✓	X	n/a		Unknown
Groen *et al.* 2011 NVALT-4[138]	Metastatic 1st line	Holland	2003 to 2007	✓	✓	Confirmed bisphosphonates not used as SOC at the time		Not used

Key: n/a, Not Applicable; NK, Not Known.

Table 15: Bisphosphonate use in metastatic NSCLC trials -2

Study	Setting	Country	Accrual period	Contacted	Responded	Response details	Relevant Clinical Guidance	Bisphosphate Co-administration
Gitlitz *et al.* 2014[142]	Metastatic 1st line	USA	2002 to 2003	✓	X	n/a	Recommended in ASCO guidance since 2003	Possible
Reckamp *et al.* 2015[143]	Metastatic 2nd line	USA	2003 to 2004	✓	✓	Confirmed, allowed as part of SOC		Likely
Edelman *et al.* 2015[144]	Metastatic 2nd line	USA	2003 to 2004	✓	X	n/a		Possible
Edelman *et al.*2017[145]	Metastatic 1st line	USA	NK	✓	X	n/a		Possible

Key: n/a, Not Applicable; NK, Not Known.

Table 16: Bisphosphonate use in metastatic breast cancer

Study	Setting	Country	Accrual period	Contacted	Responded	Response details	Bisphosphate Co-administration
Dirix *et al.* 2008[146]	Metastatic breast cancer	Multi-national	2002	✓	✓	No further data available	Possible
Falandry *et al.*2009[133] **GINECO study**	Metastatic Breast cancer	France	2003 to 2004	✓	X		Likely

2.3.5 Synthesis of results

Sufficient data was obtained to undertake a meta-analysis two disease settings, metastatic NSCLC and metastatic breast cancer, using the outcomes PFS, OS and ORR.

NSCLC

Overall, the addition of a cox-2 inhibitor did not significantly improve PFS in metastatic NSCLC (HR 0.97, CI 0.87-1.09). This is based on data extracted from 9 trials (1895 patients), representing 86% of all those randomised to the eligible trials identified. This is 96% of randomised patients with locally advanced or metastatic disease; the disease setting of most relevance to contextualising the STAMPEDE data and where PFS is a valid endpoint. All studies evaluated cox-2 inhibitors in addition to SOC however therapeutic regimes varied, including different chemotherapies or tyrosine kinase inhibitor e.g. erlotinib. Studies also differed in treatment setting and disease stage (locally advanced and/or metastatic), however despite this, was no evidence of significant statistical heterogeneity between trials (I^2 0%; p=0.62).

8 trials assessed OS, compromising 1584 patients, equivalent to 72% of all randomised patients. There is no evidence that cox-2 inhibitors improve OS (HR 0.98, CI 0.88 – 1.10) and again heterogeneity was not detected (I^2 0%; p=0.77). ORR was reported in 7 trials comprising 1366 patients which represents 62% of all those randomised. On this outcome alone, where HR>1.0 favour the experimental group, the addition of celecoxib is shown to be beneficial (HR 1.32, CI 1.05-1.66). Heterogeneity remains low, justifying the appropriateness of the fixed effect model (I^2 =15%; p=0.02); see **Figure 13** - **Figure 15**.

Figure 13: PFS outcome in metastatic NSCLC (primary outcome)

Study or Subgroup	Cox-2 inhibitor Events	Total	Control Events	Total	O-E	Variance	Weight	Hazard Ratio Exp[(O-E) / V], Fixed, 95% CI
De Ruysscher et al 2007	14	20	11	20	0.85	6.11	2.0%	1.15 [0.52, 2.54]
Edelman et al 2015	0	36	0	36	0.51	16.27	5.2%	1.03 [0.63, 1.68]
Edelman et al 2017 (CALGB 30801)	0	154	0	158	5.06	69.08	22.3%	1.08 [0.85, 1.36]
Gitlitz et al 2014	50	75	29	39	0	17.78	5.7%	1.00 [0.63, 1.59]
Gridelli et al 2007 (GECO)	0	119	0	120	0	51.83	16.7%	1.00 [0.76, 1.31]
Groen et al 2011 (NVALT-4)	0	281	0	280	-9.33	41.82	13.5%	0.80 [0.59, 1.08]
Koch et al 2011 (CYCLUS)	0	158	0	158	0.51	51.44	16.6%	1.01 [0.77, 1.33]
Lilenbaum et al 2006	0	67	0	66	-1.74	32.2	10.4%	0.95 [0.67, 1.34]
Reckamp et al 2015	0	54	0	53	-4.71	23.72	7.6%	0.82 [0.55, 1.23]
Total (95% CI)		**964**		**930**			**100.0%**	**0.97 [0.87, 1.09]**
Total events	64		40					

Heterogeneity: Chi² = 3.37, df = 8 (P = 0.91); I² = 0%
Test for overall effect: Z = 0.50 (P = 0.62)

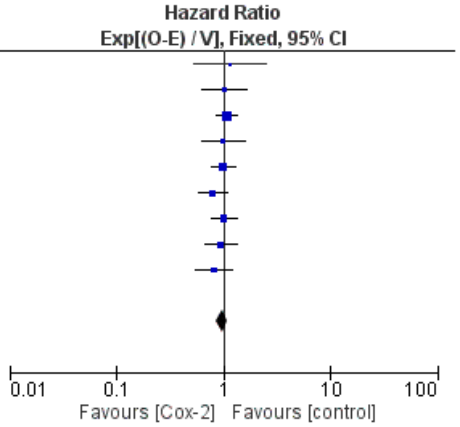


Figure 14: OS outcome in metastatic NSCLC (secondary outcome)

Study or Subgroup	Cox-2 inhibitor Events	Total	Control Events	Total	O-E	Variance	Weight	Hazard Ratio Exp[(O-E) / V], Fixed, 95% CI
Reckamp et al 2015	0	54	0	53	-2.26	19.21	6.5%	0.89 [0.57, 1.39]
Groen et al 2011 (NVALT-4)	0	281	0	280	-3.37	31.98	10.8%	0.90 [0.64, 1.27]
Gitlitz et al 2014	58	75	28	39	-2.04	19.36	6.5%	0.90 [0.58, 1.41]
Lilenbaum et al 2006	0	67	0	66	-1.74	32.2	10.9%	0.95 [0.67, 1.34]
De Ruysscher et al 2007	0	21	0	20	-0.34	8.37	2.8%	0.96 [0.49, 1.89]
Gridelli et al 2007 (GECO)	0	119	0	121	0	54.62	18.4%	1.00 [0.77, 1.30]
Koch et al 2011 (CYCLUS)	0	158	0	158	0	70.51	23.8%	1.00 [0.79, 1.26]
Edelman et al 2017 (CALGB 30801)	0	154	0	158	4.62	60.38	20.4%	1.08 [0.84, 1.39]
Total (95% CI)		**929**		**895**			**100.0%**	**0.98 [0.88, 1.10]**
Total events	58		28					

Heterogeneity: Chi² = 1.21, df = 7 (P = 0.99); I² = 0%
Test for overall effect: Z = 0.30 (P = 0.77)

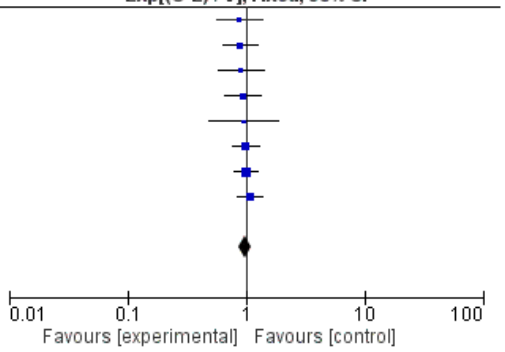
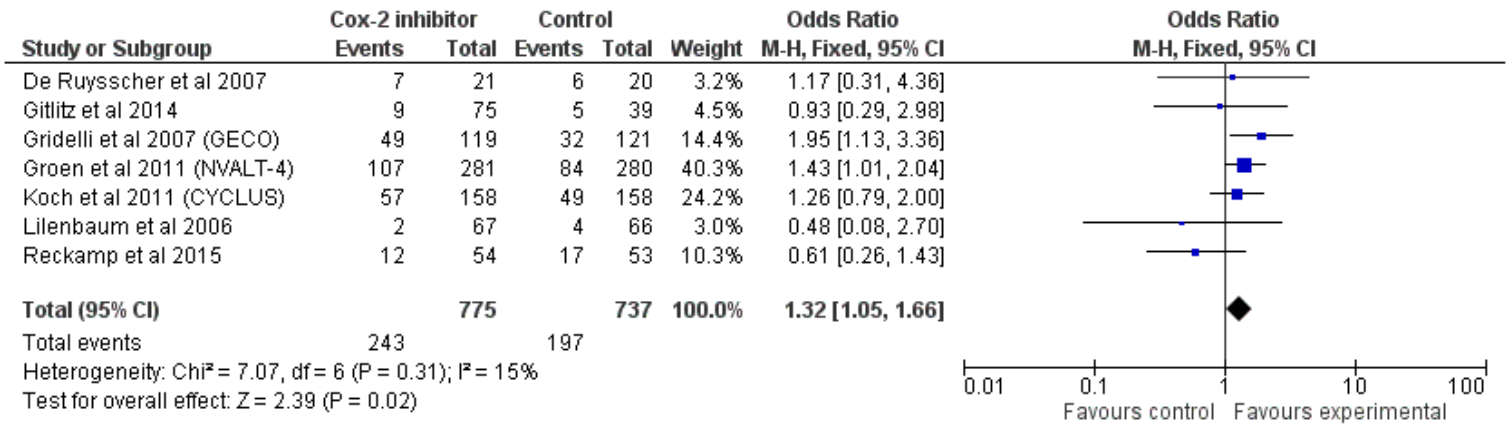


Figure 15: ORR outcome in metastatic NSCLC (secondary outcome)



Breast cancer

Sufficient data was only available for two studies in metastatic breast cancer, compromising 268 patients[133,146]. No benefit in PFS was observed (HR 0.98; 95% CI 0.76- 1.26) or ORR (HR 1.21; 95% CI 0.65-2.25). There was insufficient data to undertake a meta-analysis of OS. No statistical heterogeneity was detected for either PFS (I^2=0%; p=0.85) or ORR ((I^2=0%; p=0.55), see **Figure 16** and **Figure 17.** Both authors were contacted but no additional information was provided therefore the use of bisphosphonates is unclear.

Figure 16: PFS outcome in metastatic breast cancer (primary outcome)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	O-E	Variance	Weight	Hazard Ratio Exp[(O-E) / V], Fixed, 95% CI
Direx et al 2008	0	51	0	49	-4.06	25.09	42.3%	0.85 [0.58, 1.26]
Falandry et al 2009 (GINECO)	0	74	0	83	2.65	34.19	57.7%	1.08 [0.77, 1.51]
Total (95% CI)		**125**		**132**			**100.0%**	**0.98 [0.76, 1.26]**
Total events	0		0					

Heterogeneity: Chi² = 0.83, df = 1 (P = 0.36); I² = 0%
Test for overall effect: Z = 0.18 (P = 0.85)

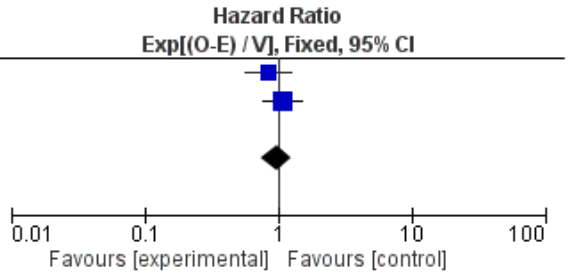
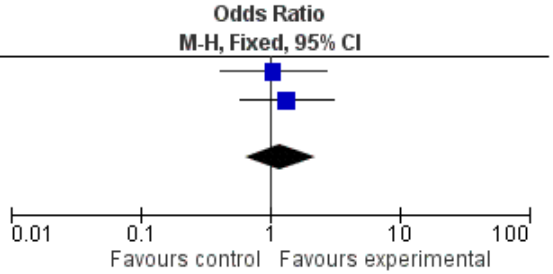


Figure 17: ORR in metastatic breast cancer (secondary outcome)

Study or Subgroup	Cox-2 inhibitor Events	Cox-2 inhibitor Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Direx et al 2008	12	51	11	49	47.4%	1.06 [0.42, 2.70]
Falandry et al 2009 (GINECO)	15	64	13	70	52.6%	1.34 [0.58, 3.09]
Total (95% CI)		**115**		**119**	**100.0%**	**1.21 [0.65, 2.25]**
Total events	27		24			

Heterogeneity: Chi² = 0.13, df = 1 (P = 0.71); I² = 0%
Test for overall effect: Z = 0.60 (P = 0.55)



Colorectal cancer

Sufficient data was available to permit meta-analysis of metastatic CRC studies, however this is the least relevant as metastatic bone involvement is rare[132,134]. No PFS benefit was shown in the two studies where this outcome was assessed, compromising 515 patients (HR 1.04; CI 0.85-1.26). The same RCT assessed OS and similarly no benefit is seen (HR 1.05; CI 0.84-1.31). No significant heterogeneity was detected between these two studies. Data from all four studies was obtained for ORR (683 patients) and results demonstrate no benefit (HR 0.65; 0.48-0.88) however there was variation detected (I^2=89%; p=0.005). When the random effects model was applied the result remains consistent, favouring control over experimental groups (HR 0.49; CI 0.14-1.66)[132,134,147,148], see **Figure 18** - **Figure 20**.

Prostate and other cancer types

There were no additional eligible trials identified other than STAMPEDE therefore it was not possible to undertake a meta-analysis. There were also insufficient data to warrant meta-analysis of other outcomes in other cancer types.

Figure 18: PFS in metastatic CRC (primary outcome)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	O-E	Variance	Weight	Hazard Ratio Exp[(O-E) / V], Fixed, 95% CI
Fuchs et al 2007	0	213	217	0	5.32	80.83	82.2%	1.07 [0.86, 1.33]
Kohne et al 2008	0	42	0	43	-1.83	17.48	17.8%	0.90 [0.56, 1.44]
Total (95% CI)		**255**		**43**			**100.0%**	**1.04 [0.85, 1.26]**
Total events	0		217					

Heterogeneity: Chi² = 0.42, df = 1 (P = 0.52); I² = 0%
Test for overall effect: Z = 0.35 (P = 0.72)

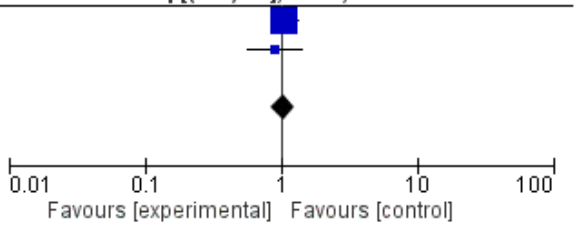


Figure 19: OS in metastatic CRC (secondary outcome)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	O-E	Variance	Weight	Hazard Ratio Exp[(O-E) / V], Fixed, 95% CI
Fuchs et al 2007	0	213	0	217	5.63	73.15	90.7%	1.08 [0.86, 1.36]
Kohne et al 2008	0	42	0	43	-1.68	7.51	9.3%	0.80 [0.39, 1.63]
Total (95% CI)		**255**		**260**			**100.0%**	**1.05 [0.84, 1.31]**
Total events	0		0					

Heterogeneity: Chi² = 0.62, df = 1 (P = 0.43); I² = 0%
Test for overall effect: Z = 0.44 (P = 0.66)

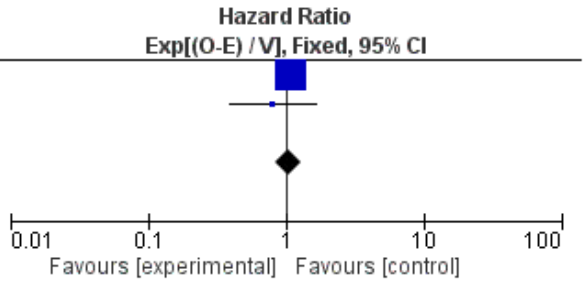
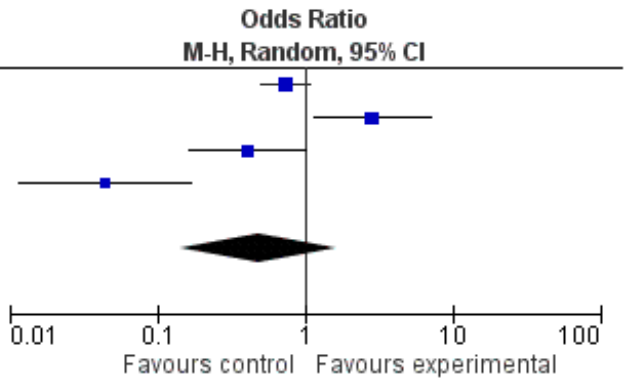


Figure 20: ORR in metastatic CRC (secondary outcome)

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Fuchs et al 2007	84	213	101	217	28.3%	0.75 [0.51, 1.10]
Jin et al 2011	38	58	12	30	25.1%	2.85 [1.15, 7.07]
Kohne et al 2008	11	42	20	43	25.1%	0.41 [0.16, 1.02]
Maiello et al 2006 (GOIM)	14	39	38	41	21.5%	0.04 [0.01, 0.17]
Total (95% CI)		**352**		**331**	**100.0%**	**0.49 [0.14, 1.66]**
Total events	147		171			

Heterogeneity: $Tau^2 = 1.33$; $Chi^2 = 27.00$, $df = 3$ ($P < 0.00001$); $I^2 = 89\%$

Test for overall effect: $Z = 1.15$ ($P = 0.25$)



2.3.6 Review of additional translational analyses

Two RCTs assessed cox-2 overexpression as a predictive and prognostic biomarker in metastatic NSCLC however evidence remains inconclusive. In a retrospective analysis of the NVALT-4 study cohort, cox-2 expression was shown to be prognostic of PFS and OS in a univariate, but not multivariate analysis. Analyses of predictive effect were inconclusive and likely underpowered as suitable tissue was only available for 167 (31%)[138]. The CALGB 30801 study reported by Edelman *et al*. 2017 evaluated apricoxib in advanced NSCLC enriched for cox-2 overexpression; randomisation was stratified by cox-2 index but again, concluded no evidence of predictive or prognostic effect for either the dichotomised or continuous variable[145].

A second approach to biomarker stratification to predict sensitivity to cox-2 inhibition is through measuring the major urinary metabolite of PGE2 (PGE-M) which, when raised may be considered a measure of cox-2 activity. Non-randomised phase II data suggests a large PGE-M decline in patients receiving cox-2 inhibitors may predict therapeutic sensitivity and has been shown to associate with improved survival[149]. This has been evaluated in two RCTs; the first randomised 109 patients; 89 provided urinary samples for additional analysis with the largest PFS improvement observed in those with high baseline urinary PGE-M. In the unselected population the median PFS improved from 3.5 to 5.4 months (HR 0.82; p=0.33); in the subgroup with high baseline urinary PGE-M this was prolonged from 2.2 months to 5.4 months (HR 0.67; p=0.15)[143]. The second RCT (APRiCOT-L) evaluated this as a tool for biomarker-selection, restricting eligibility to those patients exhibiting >50% decline in urinary PGE-M assessed following a run-in period of 5 days apricoxib treatment, however the trial failed to show a benefit for the cox-2 containing experimental arm[142].

Trials evaluating translational outcomes can help elucidate therapeutic mechanisms of action, however when studied in relatively small trials, results have been inconsistent. Two neo-adjuvant trials in breast cancer assessed pre and post-treatment cancer tissue and evaluated gene expression and Ki-67 status, as a marker of cellular proliferation. Gene expression data (n=37) demonstrate upregulation of genes functionally involved in extracellular matrix organisation, wound response, cell adhesion and biological adhesion together with receptor activator of nuclear factor kappa-B (RANK) signalling[150]. The impact on proliferation-associated genes was reflected in a significant reduction in Ki-67 in celecoxib-treated cases whilst the recruitment of tumour-infiltrating leukocytes to cancer tissue also supports the functional significance of the gene expression data. However the

second study (n=120) found no significant change in Ki-67 observed between the celecoxib and placebo groups, despite a longer duration of neo-adjuvant treatment (6 weeks).

Two PCa trials reported translational outcomes, but neither study elucidated a therapeutic mechanism of action for celecoxib given alone in the neo-adjuvant setting. In a randomised cohort of 64 men, celecoxib was not shown to be associated with significant changes in any of the biomarkers of proliferation, apoptosis or angiogenesis after 4-6 weeks of neo-adjuvant treatment. Tissue penetration also appeared poor, the mean concentration of celecoxib within prostate tissue was 0.16umol/L; approximately 50 times lower than maximal plasma concentrations[151]. In a second study of a similar design, examination of prostatectomy samples revealed no statistically significant difference in markers of apoptosis or prostaglandin levels; although this study is limited due to low accrual (28 of planned 44 patients) as the trial closed early due to safety concerns[131].

Translational data in other cancer types is limited to two studies in metastatic CRC and urothelial cancer. In a placebo-controlled RCT of 44 patients undergoing liver resection for metastatic colorectal pre-operative rofecoxib was shown to reduce microvascular density but had no significant impact on markers of apoptosis or proliferation as determined by immunohistochemistry (IHC)[152]. The only study to evidence a biological correlate for therapeutic benefit was a small placebo-controlled RCT (n=26) with high-grade invasive transitional cell carcinoma (TCC). Pre-operative celecoxib was associated with increased apoptosis and decreased tumour cell expression of Vascular endothelial growth factor (VEGF) consistent with anti-cancer effects of 2-3 weeks neo-adjuvant therapy[153].

2.3.7 Ongoing trials of interest

Several ongoing trials were identified registered on www.clinicaltrials.gov including four trials evaluating the combination of cox-2 inhibitors with immunomodulatory agents, summarised in **Table 17.**

Table 17: Ongoing trials of cox-2 inhibitors and immunomodulatory agents

Trial ID	Patient Group	Treatment	Outcomes	Sample size and trial status
NCT02268825	Advanced metastatic GI cancers	Pembrolizumab + FOLFOX6 + Celecoxib	Safety	**Design:** Phase I **Accrual:** 39 (target NK) **Status:** In follow-up **Timelines:** Est completion Jan- 2020
NCT02615574	Metastatic CRC	Type-1 Polarized dendritic cell (aDC1) + interferon-a2b + rintatolimod + celecoxib	OS	**Design:** Single arm phase II **Accrual:** 44 (target NK) **Status:** Not yet open
NCT03026140 **(NICHE Trial)**	Neo-adjuvant treatment for localised CRC	Nivolumab + Ipiliumab Nivolumab + Ipiliumab + celecoxib	Safety & Toxicity Secondary: Translational, relapse-free survival	**Design:** Randomised phase II **Accrual:** Ongoing (target 60) **Open:** Feb-2017 **Status:** Recruiting
NCT01545141	Neo-adjuvant treatment for advanced CRC suitable for surgery	Celecoxib + rintatolimod (control: observation)	Change in tumour infiltrating CD8+ cells	**Design:** Randomised phase I/II **Accrual:** Ongoing (target 50) **Status:** Recruiting **Timelines:** Est completion Dec-2020
NCT02432378	Recurrent ovarian cancer, palliative	Cisplatin + Celecoxib + Dendritic cell vaccine	Change in tumour infiltrating CD8+ cells in peritoneal fluid	**Design:** Randomised phase I/II **Accrual:** Ongoing (target 40) **Status:** Recruiting **Timelines:** Est completion Dec-2020

Summarised from www.clinicaltrials.gov who define completion as completion of data collection for the primary outcome i.e. last patient last visit.

2.4 Discussion

2.4.1 Summary of evidence

These analyses have not found any supporting clinical data for the findings of the celecoxib-ZA comparison evaluated within STAMPEDE. No other trials were identified investigating the combination of a cox-2 inhibitor with a bisphosphonate, even in extended searches including non-randomised trials. Data in PCa is very limited; STAMPEDE is the only RCT to have evaluated cox-2 inhibitors in mCSPC. Two trials identified in overlapping populations both terminated early, emphasising the impact of initial concerns regarding toxicity, which have since been shown not to be relevant to celecoxib. Therefore relevant clinical data is restricted to metastatic NSCLC or breast cancer as pre-defined disease settings of interest due to the predominance of bone involvement.

The addition of cox-2 inhibitors are not shown to benefit either indication. The level of evidence is greatest in metastatic NSCLC where data capture was high, the risk of bias was judged to be low and the treatment exposure was least affected by trial termination. Whilst no benefit is seen in PFS or OS, meta-analysis of the secondary outcome ORR is consistent with benefit and it is notable that results from the only trial confirmed to permit bisphosphonate treatment as part of per-protocol SOC, are suggestive of benefit[143].

Sufficient data was also identified to permit analyses in metastatic CRC. If, as hypothesised, the selective beneficial effect observed in the STAMPEDE data is due to synergy of bisphosphonates and a cox-2 inhibitor in the presence of metastatic bone involvement, we would not expect to observe benefit in CRC where bone involvement is rare and bisphosphonates are not used. No effect is seen on PFS, ORR or OS, with the consistency in effect with survival outcomes reassuring given the risk of bias identified in the three unblinded trials which relied upon investigator assessment of progression and response. Whilst this finding is therefore consistent with the hypothesis, the absence of benefit may also reflect the limited treatment exposure. Trials in CRC were most frequently terminated early, likely reflecting that evidence suggesting increased cardiovascular risk arose in CRC prevention trials.

The impact of cardiovascular toxicity concerns has been considerable. It has limited the acquisition of clinical data through curtailing recruitment, shortening treatment durations, increasing attrition bias through lack of reported survival data and increasing the risk of trials remaining unreported. Only 1 of the 9 terminated trials was evaluating rofecoxib, all

of the remaining were investigating celecoxib, reflecting that the cardiovascular toxicities were considered a class effect at the time, although this has been recently been disproved[103].

2.4.2 Limitations

The limitations of this meta-analysis include the uncertainty surrounding bisphosphonate use. Despite efforts to review study protocols including contacting authors, given the length of time since the trials were conducted, data remains incomplete. Based on the knowledge available, concurrent use of bisphosphonates appears uncommon. This limits the relevance of these data, but does not undermine the rationale for the original systematic review.

The planned focus was on trials in metastatic cancer, specifically including tumour types where metastatic bone involvement is frequent. However, the trial populations were heterogeneous and often grouped as locally advanced and metastatic disease, rarely giving exact proportions of patients with metastatic bone involvement; again limiting the known relevance of each trial cohort. Trials evaluating cox-2 inhibitors are at risk of having remained unpublished following the withdrawal of rofecoxib as investigators/sponsors may have deprioritised dissemination of results taking the view that safety concerns would prevent results impacting on practice. I have sought to limit the impact of this reporting bias by including review of clinical trial registries. However as highlighted by the two unpublished studies, registry data is more restricted and may be insufficient to permit further meta-analyses.

A further limitation is that cox-2 inhibitor treatment duration was limited in the majority of studies. Insufficient data is available to permit further analyses but pre-clinical data suggests the effect of cox-2 inhibitors to be dose dependent so shortened treatment durations may reduce the ability of individual trials or meta-analyses to reliably evaluate clinical response[154].

2.4.3 Exploring potential therapeutic mechanisms of action

Through inclusion of trials with translational outcomes or biomarker-defined sub-analyses, I sought to develop a better understanding of the celecoxib-ZA results. Several studies have sought to evaluate Cox-2 expression as a putative biomarker but neither prognostic or predictive effects have consistently been shown[138,144,145]. Furthermore, the lack of correlation between Cox-2 overexpression and urinary PGE-M undermines the functional

relevance of these proposed biomarkers of prostaglandin synthesis[144,155]. Of the two approaches, there is more supportive data for the measurement PGE-M, a urinary metabolite of PGE_2. Two trials have evaluated raised urinary PGE-M as a biomarker predictive of sensitivity to cox-2 inhibitors. However in both, apricoxib was given in addition to erlotinib but prior to the routine assessment of epidermal growth factor receptor (EGFR) status, which was only measured for 79% and 29% respectively. As the proportion of EGFR mutant patients on the control and experimental arm is not known this represents an important potential confounder. The APRiCOT-L trial evaluated decline in urinary PGEM hypothesised to identify a population of patients with advanced NSCLC sensitive to cox-2 inhibitors[144]. Results failed to show any survival benefit for the addition of apricoxib, however this enrichment strategy may have been undermined by uncertainly as to the most suitable cut-off value[142,149]. Therefore, neither cox-2 expression or urinary PGE appear to have clear clinical utility or provide explanation for the heterogeneity observed by metastatic status.

One hypothesis, suggested by recent pre-clinical data is that the combination of celecoxib-ZA interact to promote an anti-cancer immunological effect with the metastatic tumour microenvironment. Prostaglandin E2 has an immunological function, facilitating Th1 cell differentiation. Therefore, through inhibiting PGE2, celecoxib promotes a Th1-cytokine response, characterised by interferon-gamma (IFN-γ) and interleukin-2 (IL2) release[156,157]. ZA inhibits enzymes in the mevalonate pathway of lipid synthesis, promoting the accumulation of isopentenyl pyrophosphate (IPP), an intermediary in this pathway and a stimulator of a specific subset of regulatory T cells, γδ T cells[158,159]. This T cell subpopulation can be activated by non-MHC (major histocompatibility complex) restricted antigen presentation. This enables tumour cells to act as antigen presenting cells promoting an adaptive anti-cancer effect, whilst γδ T cells toll-like receptors (TLR) mean an innate response can also be triggered[160]. This, together with their high cytokine production ability is proposed to explain the anti-cancer effect of γδ T cells, observed *in vitro* and *in vivo*. However, clinical evidence of anti-cancer effects of γδ T cells has been contradictory with response rates ranging between 20% and 57%. This may be explained by the *in vitro* observation that different subtypes of γδ T cells have both pro and anti-cancer effects[161].

Recent data have highlighted the importance of the cytokine balance and microenvironment in determining γδ T subtype differentiation, leading to the speculation that this could be the mechanism by which celecoxib and ZA interact. In support of this, in

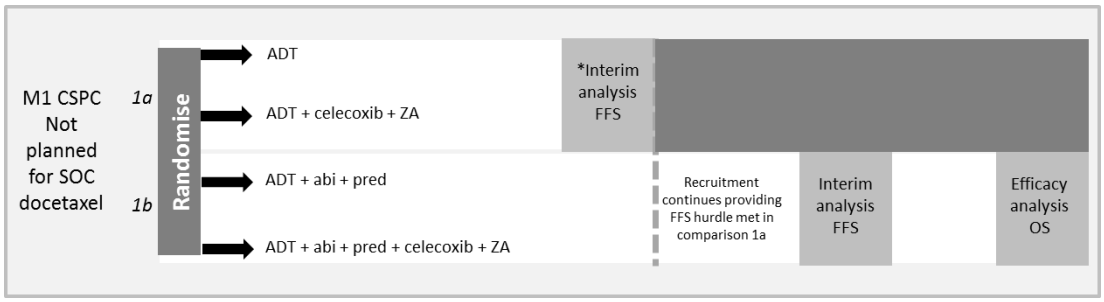
an *in vivo* model of wound healing, celecoxib has been shown to reduce the production of interleukin-17 (IL-17), recognised to be an important trigger of immunosuppression and differentiation to the pro-cancerous γδ T subtype (δ1 γδ T cells)[162,163]. Furthermore, the selective effect in men with metastatic disease supports a therapeutic interaction with tumour microenvironment. Therefore, when celecoxib triggered Th1-priming and cytokine release is combined with IPP stimulated γδ T cell function, it is speculated that this treatment combination may promote this T cell subset to differentiate to have anti-cancer potential within the context of the metastatic microenvironment. All cox-2 inhibitors are likely capable of some degree of immuno-modulation. Paracoxib has been shown to ameliorate the immunosuppressive post-operative changes with reduced elevations in IL-2, IFY-γ and IL-17 and suppression of interleukin-4 (IL-4), interleukin 10 (IL-10) and TGF-B[164]. It is noted that consistent with this a number of novel combination trials have been registered including several assessing the combination of celecoxib with immuno-modulatory agents, see **Table 17.**

2.4.4 Future work

Future work should aim to provide mechanistic data that can strengthen the STAMPEDE results and support further clinical evaluation. The immunological hypothesis presented could be tested within a small randomised phase II trial with a parallel translational study examining gamma delta T cell isolated from sampling peripheral mononuclear blood cells (PMBCs). Future clinical evaluation should aim to both validate the STAMPEDE result and ensure it can be applied within the current treatment paradigm. Having been shown to be a well-tolerated treatment, celecoxib-ZA may be considered as alternative to the addition of docetaxel. However, once accessible abiraterone is likely to become the standard-of-care for a large proportion of those not offered chemotherapy. A MAMS approach could efficiently acquire confirmatory data whilst concurrently obtaining updated data incorporating abiraterone. This could be addressed within STAMPEDE through the addition of 4 new arms, allowing two separate comparisons (shown as 1a and 1b in **Figure 21**). This design can be modelled based on the FFS effect size shown in the celecoxib-zoledronic acid M1 sub-group (HR 0.77) and median FFS observed in the M1 sub-group in the abiraterone comparison. The celecoxib-ZA comparison would reach the interim FFS analysis first and continued recruitment to the much longer, larger and more costly abiraterone containing comparison would be dependent on this result being consistent with the original STAMPEDE result. As well as ensuring these data are relevant to the current SOC, this approach may have additional patient benefit. For example the addition of a

bisphosphonate may reduce the fracture risk associated with prolonged androgen deprivation and exacerbated by abiraterone and prednisolone.

Figure 21: Proposed MAMS trial design to further evaluate celecoxib + ZA



Men with metastatic CSPC not suitable or who decline docetaxel would be eligible to be randomised 1:1:1:1. The original STAMPEDE comparison would be replicated with the co-recruitment to a longer abiraterone containing comparison being dependent on a similar FFS signal being shown.

Key: Abi, abiraterone; ADT, androgen deprivation therapy; FFS, failure free survival; pred, prednisolone; OS, overall survival; SOC, standard-of-care; M1, metastatic; ZA zoledronic acid.

2.5 Conclusions

The results of these analyses have been unable to identify external clinical data reporting similar activity for the combination of cox-2 inhibitor and bisphosphonate. Further clinical evaluation of this treatment strategy is warranted given the degree of clinical benefit. The treatment combination was well tolerated and a recent meta-analysis has confirmed that celecoxib does not confer increased cardiovascular risk, so this should not be perceived as a barrier to further evaluation. Future clinical trials should focus on patients with metastatic disease with bone involvement. Through parallel translational programmes the potential biological hypotheses for synergy such as immunomodulation and impact on the metastatic niche, particularly at the site of bone metastases, could be explored. Only through improved understanding of the mechanism of treatment heterogeneity will it be possible to determine the scope of patient benefit, which may yet be shown to be relevant to other metastatic disease settings.

Chapter 3 Prostate-specific antigen kinetics as prognostic biomarkers

3.1 Introduction

PSA is an enzyme and a member of the kallikrein-related peptidase family[165]. Clinically, it has been used since the 1980's having been shown to have a role in the detection, risk-stratification and monitoring of prostate cancer[166]. PSA is secreted by epithelial cells within the prostate gland and functions to liquefy the seminal fluid[167]. In health, PSA is retained within the prostate gland and blood levels remain low. Prostatic disease, both benign and malignant, can cause a rise a circulatory levels, with the highest levels seen in prostate cancer, thought to be due to a disruption of the tissue architecture[166]. The promoter region of kallikrein-3 (*KLK3*), the gene encoding PSA, is androgen responsive meaning PSA transcription is androgen dependent[165]. Therefore ADT initially causes a rapid PSA decline due to both tumour regression and suppression of PSA transcription[167]. The clinical utility of PSA as a marker of disease burden is therefore greatest in the castrate-sensitive setting when AR-dependent pathways are the predominant drivers of disease[168].

The relationship between early PSA response and OS will be explored as a potential on treatment biomarker that may provide early indication of risk, able to guide the use the intensified treatment strategies for mCSPC. PSA response will be defined as the proportionate change, expressed as a percentage of the baseline value, consistent with the Prostate Cancer Working Group (PCWG) criteria[169]. Previous analyses conducted in small mCSPC cohorts (n=57, 73) have demonstrated large PSA responses to ADT; 80% of patients experience a PSA response of >80% and around half will have a PSA response of >90%[170,171]. Retrospective analyses have shown that the magnitude of PSA response positively correlates with time to progression to CRPC[170-172]. Conversely, persistent PSA elevation 3 months after initiating ADT is associated with rapid progression[171]. The majority of these analyses are small with relatively short follow-up so lack power to detect survival difference, instead relying on time to treatment failure, often measured by PSA change. However as the definition of PSA progression is linked to PSA response, OS is judged the most robust outcome to determine prognostic impact. To date, the only setting where PSA response has been shown to associate with survival differences is mCRPC. Early PSA responses assessed 4 weeks after initiating abiraterone or enzalutamide have been shown to associate with OS and rPFS in multivariable models adjusted for other known prognostic

factors[173-177]. Analysis within STAMPEDE provides an opportunity to assess this in a large, sufficiently powered mCSPC cohort with mature OS data.

It is hypothesised that the prognostic value of PSA may be greatest in the castrate-sensitive setting. This is suggested by the assessment of surrogacy of PSA outcomes in mCRPC. On treatment prognostic biomarkers may be evaluated as surrogate endpoints, able to provide an early indication of treatment effect. The Prentice criteria requires the surrogate to be in the causal disease pathway and to capture the net treatment effect[178]. Abiraterone, an AR-targeted treatment, exerts an anti-cancer effect via a PSA-dependent pathway, supporting the evaluation of PSA kinetics as potential surrogates of response. In a statistical modelling analysis that pooled data from COU-301 and COU-302, two phase III trials of abiraterone in mCRPC post and pre-chemotherapy respectively, measures of PSA kinetics were used to construct a model shown to be predictive of survival[173]. For chemo-naïve patients, the effect of abiraterone on survival was no longer significant having adjusted for PSA kinetics fulfilling the Prentice criteria. The value of PSA outcomes was shown to be less in more advanced mCRPC, consistent with the molecular data demonstrating the progressive involvement of aberrant-AR and non-AR pathways [179]. For example, in an adjusted analysis, maximum PSA decline (%) was significantly associated with treatment effect in chemo-naïve patients; HR 1.38 (95% CI 1.08-1.75) p=0.01, but not chemotherapy pre-treated patients, HR 1.09 (95%CI 0.92-1.31) p=0.30[173]. Together, these data suggest that PSA outcomes are likely to best capture treatment effects in early disease, when PSA-dependent pathways are the most dominant drivers of disease progression and support the evaluation of PSA kinetics as prognostic biomarkers in the castrate-sensitive setting.

Absolute PSA levels obtained several months post-treatment have been shown to be prognostic in mCSPC treated with ADT+/- docetaxel in two secondary analyses within clinical trial cohorts[180,181]. Hussain *et al.* reported that absolute PSA assessed after a minimum of 7 months ADT was inversely associated with overall survival. The SWOG 9346 trial assessed whether survival is equivalent with intermittent versus continuous ADT. Men with mCSPC with a presenting PSA of ≥5ng/ml were eligible for registration prior to commencing ADT. Late registration was permissible in those who had commenced ADT within the previous ≤6 months (~30% of those registered). Following 7 months induction ADT those achieving a PSA ≤4ng/mL were eligible for randomisation to intermittent or continuous treatment. All registered participants were followed up for survival (n=1345). In a multivariable risk model that included performance status, Gleason score, bone pain and

PSA at study entry, patients with a PSA ≤4ng/ml but >0.2ng/ml at completion of induction were at less than one third the risk of death of those with a PSA>4ng/mL (HR 0.30; 0.24-0.38; p<0.0001). Comparative median survival times were 44 months and 13 months respectively. However, patients with a PSA <0.2ng/ml were shown to have the best prognosis, with less than one fifth the risk of death of those with a PSA>4ng/ml (HR 0.17; 0.13-0.21; p<0.0001) and median survival 75 months[181], see **Table 18.**

Absolute PSA has also been shown to be prognostic of OS in a similar secondary landmark analysis of the CHAARTED trial[180]. This trial randomised 790 men with mCSPC to receive ADT or ADT+docetaxel. 719 patients were eligible for this analysis; inclusion criteria included PSA measurement 7 months post starting ADT and sufficient follow-up data to at least this point. In a multivariable model, adjusting for treatment allocation, absolute PSA level was shown to be prognostic and results were highly consistent with those observed in the SWOG 9347 registrational cohort who were treated with ADT alone. The investigators used three categories of PSA response: 7 month PSA level <4ng/ml but >0.2ng/ml was associated with a 67% reduction in risk of death compared with a PSA>4ng/ml (HR 0.33; 0.23-0.47, p<0.001). However, the best prognosis was observed in the 7 month PSA <0.2ng/ml group; (HR 0.18; 0.12-0.28, p<0.001). A similar trend in unadjusted median survival times was observed within each treatment group (ADT alone n=361) and ADT + docetaxel (n=358), but the analysis lacked sufficient power required for a multivariable adjusted analysis by treatment group, see **Table 18.**

Table 18: Summary of analyses of absolute PSA value within the CHAARTED and SWOG 9346 clinical trials

Landmark PSA value	SWOG 9346[181]			CHAARTED[180]		
	Treatment: ADT			Treatment: ADT +/- docetaxel		
	n	Median OS	Adjusted HR (95% CI)	n	Median OS	Adjusted HR (95% CI)
≤0.2ng/ml	602 (45%)	75 months (95% CI 62-91)	HR 0.17 (0.13-0.21)	266 (37%)	60 months (95% CI 46-73)	HR 0.18 (0.12-0.28)
>0.2 ≤4ng/ml	360 (28%)	44 months (95% CI 39-55)	HR 0.30 (0.24-0.38)	214 (30%)	52 months (95% CI 40-69)	HR 0.33 (0.23-0.47)
>4ng/ml	383 (28%)	13 months (95% CI 11-16)	HR 1.0 (reference)	239 (33%)	22 months (95% CI 20-27)	HR 1.0 (reference)

As opposed to using a pre-specified 'landmark reading', PSA nadir (defined as the lowest PSA measurement on treatment) has also been proposed as an on treatment biomarker, and shown to be prognostic of survival in ADT-treated mCRPC. The best evidence for this comes from analyses of the STAMPEDE control group. The trial protocol requires PSA measurements 6 weekly until 6 months post randomisation, from which the nadir value is calculated, defined as the lowest value reported during this time period. In an analysis (n=917) of men with mCSPC allocated to receive ADT alone between 2005 and 2013, PSA nadir≥4ng/ml was associated with an increased risk of death compared with those achieving PSA nadir< 4ng/ml (HR 2.43; 1.85-3.19, p<0.001)[182]. This confirms the association previously demonstrated in smaller cohorts and retrospective reviews, where PSA nadir has been shown to be prognostic of improved PFS, with similar trends observed in survival[172,183,184].

As yet, PSA nadir defined in this way has not been examined in patients receiving the updated SOC, ADT + docetaxel. Given the observed variability in survival outcomes (see **Table 3**) there is a need to identify patients who remain at high-risk of poor survival outcomes despite docetaxel. PSA nadir is a potential on treatment biomarker that could reflect response to ADT+ docetaxel and provide prognostic information at a clinically meaningful landmark which correlates with docetaxel completion. This could be used to identify a population in whom to evaluate additional strategies within a clinical trial. In the future, should the combination of ADT + docetaxel + AR-targeted therapy be shown to be beneficial (currently under evaluation in several trials including PEACE-1 and ENZAMET, see **Table 1**, this potential biomarker could be used to risk-stratify and identify a population in whom the additional toxicity and costs may be justified.

3.1.1 Research aims

In this chapter I will address the following:

Research questions

- Is the magnitude of PSA response prognostic of OS?
- Is PSA nadir assessed following completion of docetaxel prognostic of OS?

Hypotheses

- A large PSA response may identify a good prognostic hormone-sensitive subgroup in whom the toxicities of additional treatments may be avoided
- A high PSA nadir assessed following completion of docetaxel may identify a high-risk subgroup in whom additional therapies should be evaluated

3.2 Methods

Both research questions can be addressed through analyses of participants with metastatic disease at trial entry allocated to the STAMPEDE docetaxel-containing randomisations. In total, 1451 metastatic patients were randomised to the control group (arm A) or one of the docetaxel-containing treatment groups (arm C or arm E). As the addition of ZA to docetaxel did not impact on survival, patients allocated to either arm C or E will be combined[20]. This accounts for the allocation ratio (2:1:1:1:1:1) and means control and docetaxel groups are of equal size; 724 metastatic control (ADT alone) and 727 metastatic patients allocated to receive ADT + docetaxel, see **Figure 4** for an overview of research arms. This analysis will focus on the patients with metastatic disease as the population of interest in this thesis. STAMPEDE also recruits high-risk M0 however, as absolute PSA level may reflect disease burden separate analyses of PSA-outcomes would be required in this disease setting as prognostic PSA thresholds may differ.

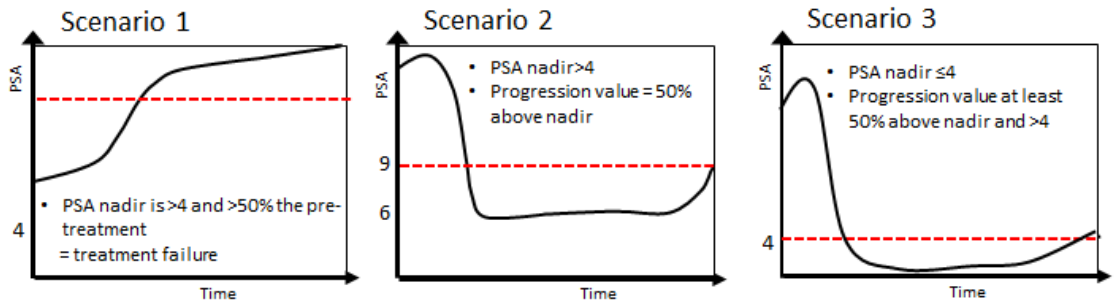
A data release request was approved by the STAMPEDE Trial Management Group (TMG) and Trial Steering Committee (TSC) allowing access to the published dataset used for primary analysis of the docetaxel-containing comparisons, see **Appendix E**. This includes data collected from participants randomised between October 2005 and March 2013 allocated to control or research arm C or E. The data were frozen on May 13th 2015. Baseline data included patient demographics, disease stage, metastatic distribution, date of

diagnosis and date of randomisation were provided, in addition to all PSA values obtained according to the protocol until 24 weeks post randomisation (6, 12, 24 weeks and the calculated nadir value). Outcome data for both OS and FFS were also obtained.

3.2.1 Outcome measures

The trial protocol defines OS as the time from randomisation to death from any cause and FFS as the time from randomisation to evidence of at least one of the following: biochemical failure, disease progression, or death from PCa. The protocol definition of biochemical failure is a relative assessment made between the pre-treatment PSA and PSA nadir. Biochemical failure is defined as immediate (PSA nadir >50% the pre-treatment PSA), or when the PSA reaches >50% above the nadir, or if the nadir value is <4ng/ml, when the PSA reaches >50% nadir and is above 4ng/ml, whichever is greater. As FFS is predominantly driven by biochemical failure, OS is preferred over FFS as the outcome for prognostic analyses of PSA kinetics, as the latter is confounded due to the definition of FFS being linked to pre-treatment and nadir PSA values; see **Figure 22.**

Figure 22: STAMPEDE trial definition of biochemical failure (illustrative examples)



Scenario 1: Immediate treatment failure PSA nadir >4ng/mL and >50% pre-treatment PSA

Scenario 2: PSA progression value 50% above pre-treatment value as PSA nadir >4ng/mL

Scenario 3: PSA progression value >4ng/L and >50% above nadir as PSA nadir ≤4ng/mL

PSA outcome definitions:

- **PSA response** is defined for the purposes of this analysis as the percentage fall in PSA from the pre-treatment value to the first post-ADT value obtained by the protocol (6-weeks post randomisation).

  PSA response (%) = (pre-treatment PSA-week 6 PSA/ pre-treatment PSA) x 100

- **PSA nadir** is as defined by the STAMPEDE protocol, the lowest PSA value reported in the first 24 weeks post randomisation.

3.2.2 Statistical approach

I undertook all statistical analyses supported by Matthew Nankivell and Matthew Sydes, statisticians at the MRC CTU at UCL. Analyses were performed using Stata version 15.0 (StataCorp LP, College Station, TX, USA) using standard survival-analysis methods; Kaplan-Meier estimates were used to produce survival curves. In all analyses multivariable models for both outcomes OS and FFS were built to include all pre-defined subgroups, including all those shown to be prognostic in a cohort analysis of the STAMPEDE control arm[182]. A p value was calculated using the likelihood ratio to assess the impact of the variable in the survival model. All baseline characteristics that were statistically significant at the 5% level were included in the multivariable model. The PSA outcome measures were added to each separate baseline model for OS and FFS outcomes in an adjusted Cox regression analysis.

3.2.2.1 Analysis of PSA response

Population of interest

Initially, I planned to assess PSA response shortly after commencing ADT in patients randomised to the control and docetaxel-treated groups in order to explore if the magnitude of benefit from chemotherapy differed according to PSA response. Unfortunately, a lack of comparable PSA data prevented this from being possible as the STAMPEDE protocol does not collect PSA at the time of randomisation; the 6-week post randomisation value is the first post ADT PSA value collected. The vast majority of participants allocated to receive docetaxel had commenced treatment prior to this landmark; the median time to starting was 2.4 (IQR 1.4-3.7) weeks and by 6-weeks, over 50% had received 2 cycles. Therefore the impact of PSA response was explored separately in the groups allocated to receive ADT alone (control) and ADT+docetaxel, with the latter being of primary relevance to the main hypothesis. In addition, the following two criteria were required to be eligible for inclusion:

- Metastatic disease at randomisation
- PSA data available pre-treatment and 6-weeks post randomisation to permit calculation of PSA response

Survival analyses

For the analysis of PSA response a landmark approach was used at 6-weeks post randomisation, from which both time-to-event outcomes were timed, reflecting the time point at which PSA response was assessed. The distribution of PSA response was assessed in the eligible population and the impact of the categorised variable explored in a multivariable Cox model to allow the calculation of an adjusted HR incorporating all other prognostic factors that remained statistically significant at the 5% level.

Exploring PSA response threshold values

A threshold value that identifies a good prognostic subgroup in patients who have been exposed to ADT was sought. Calculation of a concordance index (c-index) was used to measure the predictive discrimination of each threshold value. The c-index estimates the probability of concordance between predicted and observed responses, calculated by considering all pairs of patients where at least one has experienced the event (in this case death). Where the predicted survival time is longer for the patient with a longer observed survival time the pair are judged concordant and vice versa. A value 1.0 is perfect discrimination whereas 0.5 indicates no predictive discrimination of patients with different outcomes. The Harrell-C index will be calculated and the threshold with the highest discriminating value used when categorising PSA response[185,186].

Sensitivity analyses

A sensitivity analysis excluded patients with a low presenting PSA. The rationale was that those patients with metastatic disease who present with a low PSA may be considered not to have the biomarker of interest (PSA), impacting on the prognostic significance of a relative PSA change. The cut-off value of less than 15ng/ml was pre-specified as this was judged sufficiently atypical at presentation of metastatic disease.

3.2.2.2 Analysis of PSA nadir

Population of interest

The population relevant to this analysis are those allocated to receive docetaxel in research arms C or E, limited to the per-protocol population. This excludes 12% who did not report starting docetaxel; reasons included treatment refusal, patient choice and trial withdrawal[20]. The population is limited to those who can still be described as castrate-sensitive at 24-weeks post randomisation, the timing of the landmark analysis i.e. have not yet reported a FFS event (biochemical, radiological or clinical progression). The rationale

being that if progression to CRPC has already occurred additional treatment with any one of the multiple, licenced options currently available will likely be initiated reducing the need for an additional prognostic biomarker. Where there is current uncertainty and a greater need to risk-stratify is in the management of metastatic castrate-sensitive disease, the selected population of interest. Additionally, initiation of second-line treatment may affect the PSA nadir value which may no longer be considered reflective of response to first-line therapy. Therefore the selection criteria for this analysis can be summarised as:

- Metastatic at randomisation
- Allocated to a docetaxel-containing research arm (C or E)
- Commenced docetaxel treatment (per-protocol population)
- Sufficient PSA data to calculate nadir value
- No reported FFS event prior to 24 weeks post randomisation

Threshold values

The distribution of PSA nadir was explored employing categorisation as previously described in the literature[180-182]. Additional threshold values were also explored and assessed visually through plotting Kaplan-Meier graphs.

Survival analyses

For the analysis of PSA nadir a landmark of 24 weeks post randomisation was used and all time-to-event outcomes were adjusted to account for this. Similarly, a multivariable model considering the same baseline factors was built for the primary outcome of OS. Prognostic significance was assessed using the likelihood ratio and results considered statistically significant at the 5% level.

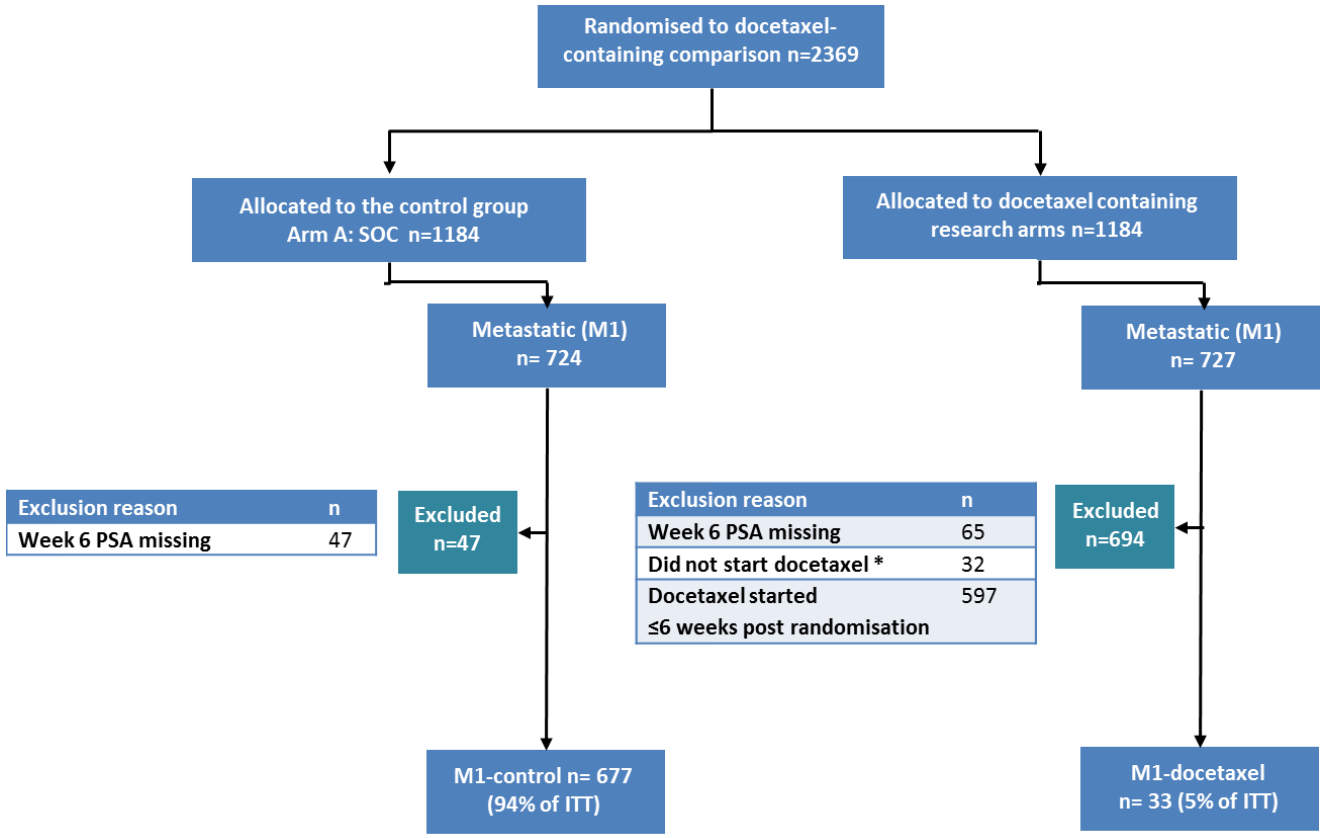
3.3 Results

3.3.1 Patient cohorts for analysis of PSA response

The cohort selection is shown in **Figure 23**. Of 724 metastatic patients allocated to the control arm within the docetaxel comparison, 677 (94%) were eligible for inclusion in this analysis. 47 were excluded due to missing PSA data at 6-weeks. The median duration of ADT prior to the assessment of PSA response was 12.3 weeks (IQR 9.1-15.0). Comparison with the metastatic intention-to-treat population (ITT) revealed a similar distribution of baseline characteristics, suggesting the analysis population were representative, see **Table 19.** The median follow-up for the analysis population was 29 months post landmark (6-weeks post randomisation).

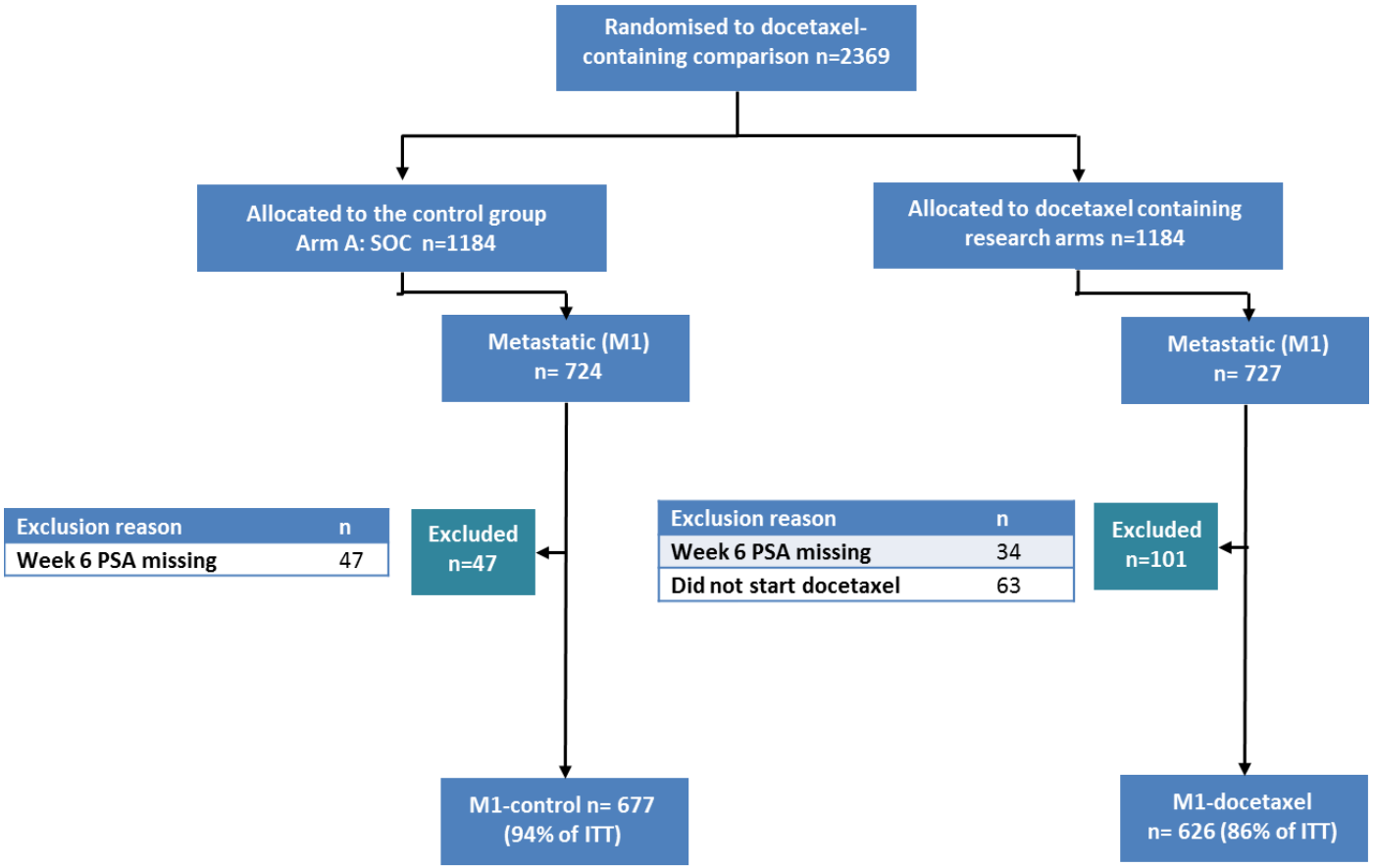
The impact of PSA response was explored separately in the docetaxel-treated cohort, see **Figure 24**. Of 727 metastatic patients allocated to receive ADT+docetaxel, 626 were eligible for inclusion (86% of ITT). 63 were excluded as docetaxel was never reportedly started, week-6 PSA was missing in a further 34 and duration of prior ADT was missing for one patient.

Figure 23: Patient selection for analysis of PSA response-1



Patients with metastatic (M1) disease at trial entry who were allocated to receive ADT alone were included if week-6 PSA was recorded. To be comparable, patients with M1 disease allocated to receive docetaxel had be in the subsequent per-protocol population (i.e. commence treatment) but may not have started it by the landmark when early PSA response was to be assessed. Only 33 patients (5% of ITT) met these criteria, limiting this analysis to those allocated to receive ADT alone.

Figure 24: Patient selection for analysis of PSA response-2



In a separate analysis, the impact of early PSA response was explored in the M1 patients allocated to receive docetaxel accepting that as docetaxel treatment had started in the majority, this population is not comparable with the ADT alone treated group. 636 patients were eligible for this analysis; 34 were excluded due to missing week-6 PSA measurements and 63 did not report commencing docetaxel. Reasons for not starting included: refusal, trial withdrawal and clinician decision and in one case, disease

Table 19: Comparative baseline characteristics in the PSA response cohort (ADT alone)

Baseline characteristics	Metastatic ITT	PSA response ADT alone treated cohort	Excluded
	n=724	**n=677**	**n=47**
Tumour stage			
≤T2	90 (12%)	85 (13%)	5 (11%)
T3	404 (56%)	380 (56%)	24 (51%)
T4	163 (23%)	154 (23%)	9 (19%)
Tx	67 (9%)	58 (9%)	9 (19%)
Nodal stage			
N0	242 (33%)	228 (34%)	14 (30%)
N+	416 (57%)	387 (57%)	29 (62%)
Nx	66 (9%)	62 (9%)	4 (9%)
Metastatic distribution			
Bone only	454 (63%)	428 (63%)	26 (55%)
Distant nodes only	63 (9%)	56 (8%)	7 (15%)
Bone & nodes or other	207 (29%)	193 (29%)	14 (30%)
Gleason sum score			
≤ 7	156 (22%)	148 (22%)	8 (17%)
≥ 8	476 (66%)	445 (66%)	31 (66%)
Unknown	92 (13%)	84 (12%)	8 (17%)
Age			
≤55	70 (10%)	9 (19%)	9 (19%)
55-59	104 (14%)	8 (17%)	8 (17%)
60-64	146 (20%)	11 (23%)	11 (23%)
65-69	187 (26%)	6 (13%)	6 (13%)
70-74	134 (19%)	10 (22%)	10 (21%)
≥ 75	83 (11%)	3 (6%)	3 (6%)
Performance status			
0	521 (72%)	495 (73%)	26 (55%)
1 or 2	203 (28%)	182 (27%)	21 (45%)
Presenting PSA (ng/ml)			
≤20	125 (17%)	116 (17%)	9 (19%)
21-50	121 (17%)	112 (17%)	9 (19%)
51-100	114 (16%)	108 (16%)	6 (13%)
101-250	143 (20%)	140 (21%)	3 (6%)
>250	221 (31%)	201 (30%)	20 (43%)
Duration of ADT prior to 6-week assessment of PSA response			
≤6 weeks	44 (6%)	43 (6%)	1 (2%)
≤10 weeks	196 (27%)	183 (27%)	13 (28%)
≤14 weeks	238 (33%)	222 (33%)	16 (34%)
≤18 weeks	239 (33%)	224 (33%)	15 (32%)
≤20 weeks	6 (1%)	5 (1%)	1 (2%)
>20 weeks	1 (0.1%)	0 (0%)	1 (2%)

3.3.2 PSA response in ADT alone group

Distribution

The median PSA response 6-weeks post randomisation, equivalent to 12.3 weeks (IQR 9.1-15.0) post-initiation of ADT, was 97% (IQR 91-99%). A PSA response of >50% was reported in 95% of the cohort, whilst only 13% reported a PSA response of <80%. Failure to respond (6-week PSA value>pre-treatment value) was reported in 2 cases within the population with sufficient PSA data to be eligible for inclusion.

Baseline survival model -1

The prognostic significance of seven baseline characteristics was evaluated in a cox regression survival model: tumour and nodal stage, metastatic distribution, Gleason score, presenting PSA, age and performance status at randomisation and prior duration of ADT. These were selected based on the published literature demonstrating prognostic significance in an analysis with the M1 subgroup allocated to the STAMPEDE control arm (n=917)[182]. Models with and without each variable were compared using the likelihood-ratio test. As shown in **Table 21** metastatic distribution, Gleason sum score, baseline age, performance status were statistically significant at the 5% level.

Baseline survival model -2

As shown in **Table 20,** the groups defined by PSA response significantly differed in two ways, patients with a PSA response <80% were more likely to have a poorer baseline performance status and patients with a PSA response ≥99% had a lower absolute PSA value at the landmark (6-weeks post randomisation). Therefore a second survival model was fitted, in which presenting PSA was removed and week 6 PSA was considered instead. This is because the two variables were shown not to be independent of each other. In this second baseline model metastatic distribution, Gleason sum score, baseline age, performance status and week-6 PSA were shown to remain statistically significant, see **Table 22.** A separate model for FFS was fitted using the same approach. The same four prognostic characteristics remained statistically significant, see **Appendix A3: Table 68**.

Defining groups based on identified threshold value

The c-stat was calculated aiming to split the group into three categories defined by PSA response. The lowest threshold was set and the c-stat was calculated for a range of upper values, adjusting for the baseline characteristics within the baseline survival model (2), see **Graph 1.** Concordance was highest when PSA response was categorised as low (<80%)

moderate (90-99%) and high ≥99%. The impact of setting different lower thresholds was explored; see **A2**: **Figure 38** - **A2**: **Figure 40.**

Table 20: Baseline characteristics by PSA response category

	PSA response ≥99%	PSA response 80 -98%	PSA response <80%	P*
	n=188 (28%)	**n=398 (59%)**	**n=91 (13%)**	
Tumour stage				
≤T2	27 (14%)	45 (11%)	13 (14%)	0.3811
T3	91 (48%)	241 (61%)	48 (58%)	
T4	49 (26%)	84 (21%)	21 (23%)	
Tx	21 (11%)	28 (7%)	9 (10%)	
Nodal stage				
N0	70 (37%)	129 (32%)	29 (32%)	0.7488
N+	98 (52%)	236 (59%)	53 (58%)	
Nx	20 (11%)	33 (8%)	9 (10%)	
Metastatic distribution				
Bone only	128 (68%)	240 (60%)	60 (66%)	0.2728
Distant nodes only	10 (5%)	40 (10%)	6 (7%)	
Bone & nodes or other	50 (27%)	118 (30%)	25 (27%)	
Gleason sum score				
≤ 7	49 (26%)	83 (21%)	16 (18%)	0.9290
≥ 8	108 (57%)	271 (68%)	66 (73%)	
Unknown	31 (16%)	44 (11%)	9 (10%)	
Age				
≤55	14 (7%)	32 (8%)	15 (16%)	0.1683
55-59	20 (11%)	64 (16%)	12 (13%)	
60-64	36 (19%)	86 (22%)	13 (14%)	
65-69*	58 (31%)	98 (25%)	25 (27%)	
70-74	33 (18%)	76 (19%)	15 (16%)	
≥75	27 (14%)	42 (11%)	11 (12%)	
Performance status				
0	141 (75%)	299 (75%)	55 (60%)	**0.0137**
1 or 2	47 (25%)	99 (25%)	36 (40%)	
Duration of ADT prior to week-6 assessment of PSA response				
≤6 weeks	8 (4%)	29 (7%)	6 (7%)	0.3255
≤10 weeks	50 (27%)	111 (28%)	22 (24%)	
≤14 weeks ***	62 (33%)	130 (33%)	30 (33%)	
≤18 weeks	65 (35%)	127 (32%)	32 (35%)	
≤20 weeks	3 (2%)	1 (0.3%)	1 (1%)	
PSA at landmark (6-weeks post randomisation)				
<0.5 ng/ml	77 (41%)	37 (9%)	1 (1%)	**0.0001**
≥0.5 to <2.5 ng/ml	70 (37%)	153 (38%)	5 (5%)	
≥2.5 to <10 ng/ml	27 (14%)	111 (28%)	16 (18%)	
≥10 to <50 ng/ml	13 (7%)	62 (16%)	28 (31%)	
≥50 ng/ml	1 (0.5%)	35 (9%)	41 (45%)	

* Kruskal Wallis test of difference by group; bold indicates result statistically significant at 5% level
* Contains median age in all groups (65 years if PSA response ≥99% or <80%, 66 years if PSA 80-90%)
** Median value prior ADT 12 weeks

Table 21: Baseline multivariable OS model for PSA response analysis (model 1)

Characteristic				Overall survival	
	Category description	**n**	**Events**	**Multivariable**	**p-value***
T stage	≤T2	85	32	0.90 (0.61-1.32)	
	T3	380	176	1.0	
	T4	154	74	1.10 (0.84-1.45)	
	Tx	58	35	1.24 (0.85-1.83)	0.5569
Nodal stage	N0	228	100	1.0	
	N+	387	184	1.09 (0.82-1.44)	
	Nx	62	33	1.04 (0.70-1.59)	0.8475
Metastatic distribution	Bone only	428	205	3.01 (1.64-5.54)	
	Distant nodal only	56	11	1.0	**<0.0001**
	Bone & nodal or other	193	101	3.47 (1.85- 6.52)	
Gleason sum score	≤ 7	148	51	1.0	
	≥ 8	445	217	1.95 (1.43-2.68)	
	Unknown	84	49	1.93 (1.25- 2.97)	**0.0005**
Age	≤55	61	38	2.15 (1.45-3.19)	
	55-59	96	56	1.56 (1.10-2.21)	
	60-64	135	65	1.08 (0.77-1.51)	
	65-69**	181	78	1.0	
	70-74	124	44	0.81 (0.55-1.17)	
	≥ 75	80	36	1.11 (0.74-1.65)	**0.0002**
Performance status	0	495	207	1.0	
	1 or 2	182	110	1.88 (1.49-2.38)	**<0.0001**
Duration of prior ADT (weeks)	Continuous	-	-	0.99 (0.96-1.02)	0.5538
Ln presenting PSA	Continuous	-	-	1.01 (0.95-1.08)	0.7168

*For variable in overall survival model, statistically significant at 5% level shown in bold

** Median age 65 years

Table 22: Baseline multivariable OS model for PSA response analysis (model 2)

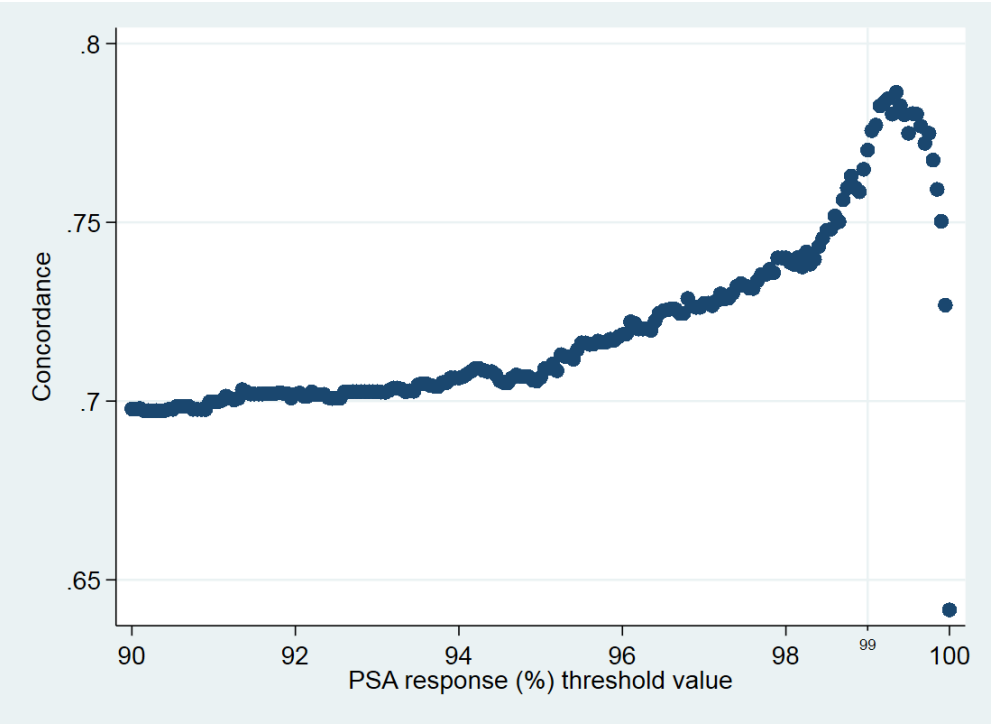
Characteristic			Overall survival		
	Category description	**n**	**Events**	**Multivariable**	**p-value***
Tumour stage	≤T2	85	32	0.96 (0.64-1.41)	
	T3	380	176	1.0	
	T4	154	74	1.06 (0.81-1.39)	
	Tx	58	35	1.13 (0.77-1.66)	0.8945
Nodal stage	N0	228	100	1.0	
	N+	387	184	1.07 (0.81-1.41)	
	Nx	62	33	0.98 (0.66-1.46)	0.8673
Metastatic distribution	Bone only	428	205	2.98 (1.62- 5.49)	
	Distant nodal only	56	11	1.0	**<0.0001**
	Bone & nodal or other	193	101	3.10 (1.65- 5.83)	
Gleason sum score	≤ 7	148	51	1.0	
	≥ 8	445	217	1.83 (1.34-2.51)	
	Unknown	84	49	1.44 (0.96- 2.18)	**0.0003**
Age	≤55	61	38	1.65 (1.11-2.46)	
	55-59	96	56	1.40 (0.99-2.00)	
	60-64	135	65	1.10 (0.80-1.53)	
	65-69**	181	78	1.0	
	70-74	124	44	0.78 (0.53-1.13)	
	≥ 75	80	36	1.19 (0.80-1.78)	**0.0125**
Performance status	0	495	207	1.0	
	1 or 2	182	110	1.75 (1.38-2.21)	**<0.0001**
Duration of prior ADT (weeks)	Continuous	-	-	0.99 (0.96-1.02)	0.4482
Ln week 6 PSA***	Continuous	-	-	1.24 (1.17-1.32)	**<0.0001**

*For variable in overall survival model, statistically significant at 5% level shown in bold

** Median age 65 years

*** Natural logarithmic transformation

Graph 1: PSA response threshold value in ADT alone group (concordance statistic)



Harroll's C-statistic was calculated for a range of threshold values and the highest predictive discriminatory value was observed for PSA response ≥99% (marker). This calculation was made using a lower threshold value of <80% which was observed to result in the highest level of concordance for the upper value. See Appendix A for calculations using alternative lower thresholds 85% and 90%.

3.3.3 Impact of PSA response on overall survival

The magnitude of PSA response was shown to be associated with improved OS (p<0.0001). When categorised, a PSA response ≥99% was associated with a reduced risk of death, with 73% 3 year survival compared with 61% if the PSA response was 80-98% and 23% if the PSA response <80%, see **Graph 2.** In the multivariable analysis this remained statistically significant using model 1, adjusting for metastatic distribution, baseline performance status, age and Gleason score; see **Table 23**. However, when absolute PSA was also adjusted for in model 2, the additional prognostic effect of PSA response was less, consistent with both the relative change and absolute PSA value being clinically significant, see **Table 24.**

Graph 2: Overall survival by PSA response in ADT alone group

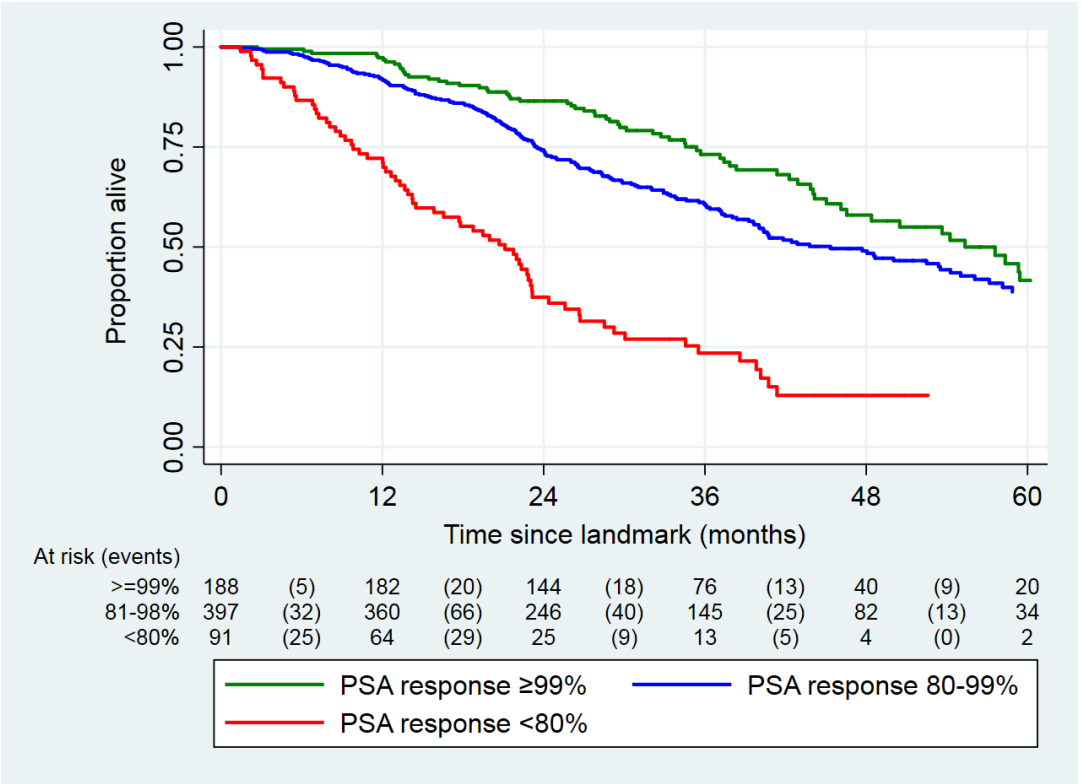


Table 23: Summary survival statistics (model-1)

PSA response	n	Events	*Adjusted HR (95%CI)	Median OS (m) (95% CI)	3yr OS	P value
≥99%	188	69	0.66 (0.50-0.88)	58 (46-66)	73%	**<0.0001**
80-98%	398	180	1.0 (reference)	45 (40-54)	61%	
<80%	91	68	2.94 (2.20-3.93)	21 (14-23)	23%	

*HR adjusted for metastatic distribution, baseline performance status, age and Gleason score.

Table 24: Summary survival statistics (model-2)

PSA response	n	Events	*Adjusted HR (95%CI)	Median OS (m) (95% CI)	3yr OS	P value
≥99%	188	69	0.79 (0.59-1.07)	58 (46-66)	73%	**<0.0001**
80-98%	398	180	1.0 (reference)	45 (40-54)	61%	
<80%	91	68	2.23 (1.61-3.08)	21 (14-23)	23%	

* HR adjusted for metastatic distribution, Gleason score, baseline age and performances status and absolute PSA at landmark (week-6 PSA). Median OS and survival rates timed from landmark 6-weeks after randomisation which equates to 12 weeks (median) post initiation of ADT

Sensitivity analysis

Consistency of effect was observed in the sensitivity analysis in which 12% of patients presenting with metastatic disease and PSA <15ng/ml were excluded. Results show patients with a PSA response of ≥99% have improved 3 year survival rates compared with those with moderate or low PSA responses. However, in the multivariable analysis the estimated prognostic improvements is less, and when adjusted for absolute PSA value this is not statistically different to the moderate PSA response group; HR 0.83; (95% CI 0.60-1.15) p<0.0015, see **Graph 3** and **Table 25**. As shown in **Table 26**, as anticipated, those with a low presenting PSA are less likely to experience a large PSA response, therefore the sensitivity analysis proportionately excludes most patients from the PSA response <80% category.

Graph 3: Overall survival by PSA response group in sensitivity population

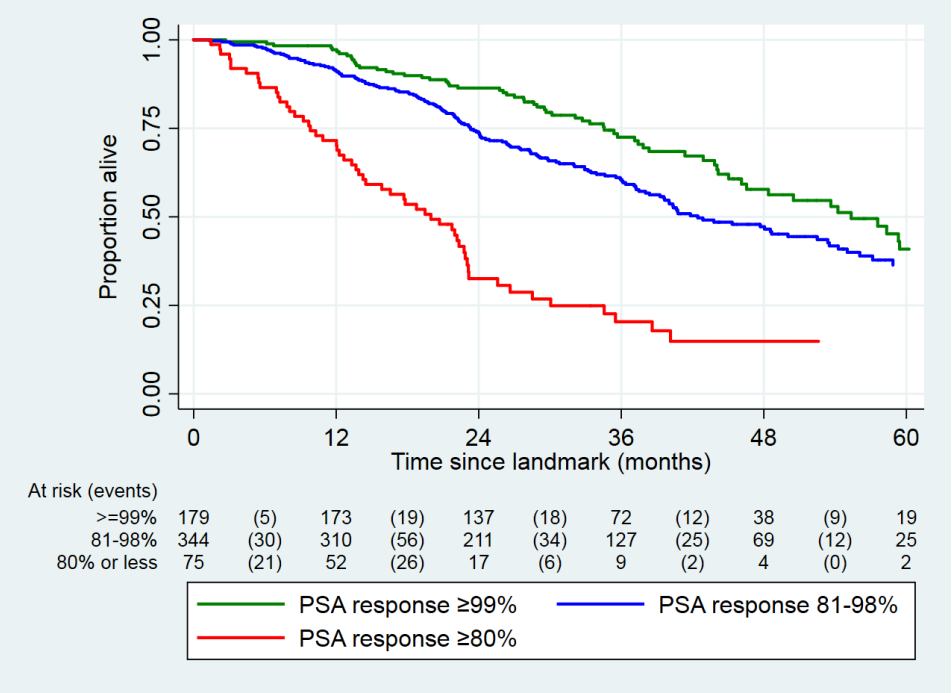


Table 25: Summary of PSA response and survival outcome in sensitivity analysis

PSA response	n	Events	Adjusted HR* (95% CI)	Median OS (m) (95%CI)	3yr OS	P value
≥99%	179	67	0.83 (0.60-1.15)	55 (46-66)	73%	**0.0015**
80-98%	345	160	1.0 (reference)	42 (39-53)	61%	
<80%	75	55	1.92 (1.33-2.77)	20 (14-23)	20%	

* Adjusted for metastatic distribution, baseline performance status, age, Gleason score and absolute PSA at landmark (week-6 PSA)

Table 26: Comparative population included in sensitivity analysis

PSA response	Primary analysis	Sensitivity analysis	Proportion excluded (% primary analysis population)
≥99%	188	179	9(5%)
80-98%	398	345	53 (13%)
<80%	91	75	16 (18%)
Totals	677	599	78 (12%)

3.3.4 Impact of PSA response on failure-free survival in ADT alone group

In a multivariable model adjusted for metastatic distribution, Gleason score, week-6 PSA and age and performance status, PSA response was shown to be prognostic of FFS (p<0.0024). 2-year FFS was 60%, 49% and 24% if the PSA response was ≥99%, 80-98% or <80% respectively.

Graph 4: Failure free survival by PSA response category

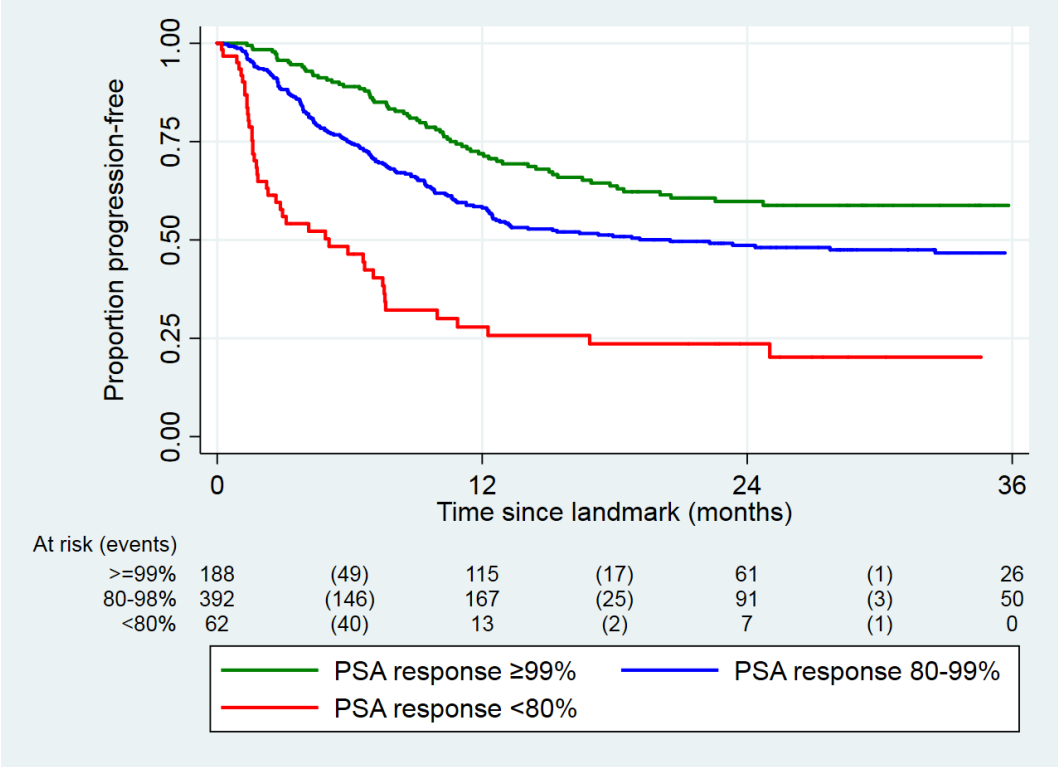


Table 27: Summary of PSA response and failure-free survival

PSA response	n	Events	*Adjusted HR (95%CI)	Median FFS (m) (95% CI)	2 yr FFS	P value
≥99%	188	132	HR 0.81 (0.60-1.10)	NR (40 - NR)	60%	**0.0024**
80-98%	398	327	HR 1.0 (reference)	21 (13 - 40)	49%	
<80%	91	86	HR 1.85 (1.28-2.67)	3 (2 - 8)	24%	

*HR adjusted for metastatic distribution, week-6 PSA, and baseline age and performance status. Median FFS timed from landmark (6-weeks post randomisation)

3.3.5 Impact of PSA response in ADT+docetaxel treated group

Distribution

The median PSA response 6-weeks post randomisation was 98% (IQR 94-.0 – 99.5%). The median absolute value was 1.6ng/ml (IQR 0.4-6.2ng/ml). Only eight patients had not experienced a PSA response. The Kruskal-Wallis test showed that both PSA response (p=0.0001) and absolute week-6 PSA (p=0.0001) were statistically significantly different between ADT alone and ADT+docetaxel treatment groups.

Table 28: Comparative PSA distribution by treatment group

	ADT alone	ADT+docetaxel	p value
PSA response (%)	97% (91.1-99.1)	98.1 (IQR 94-.0 – 99.5)	**0.0001**
Absolute PSA at landmark (ng/ml)	2.4 (0.7-11)	1.6 (IQR 0.4-6.2)	**0.0001**

*Kruskal-Wallis test of difference, statistical significant values at 5% shown in bold

Baseline survival model

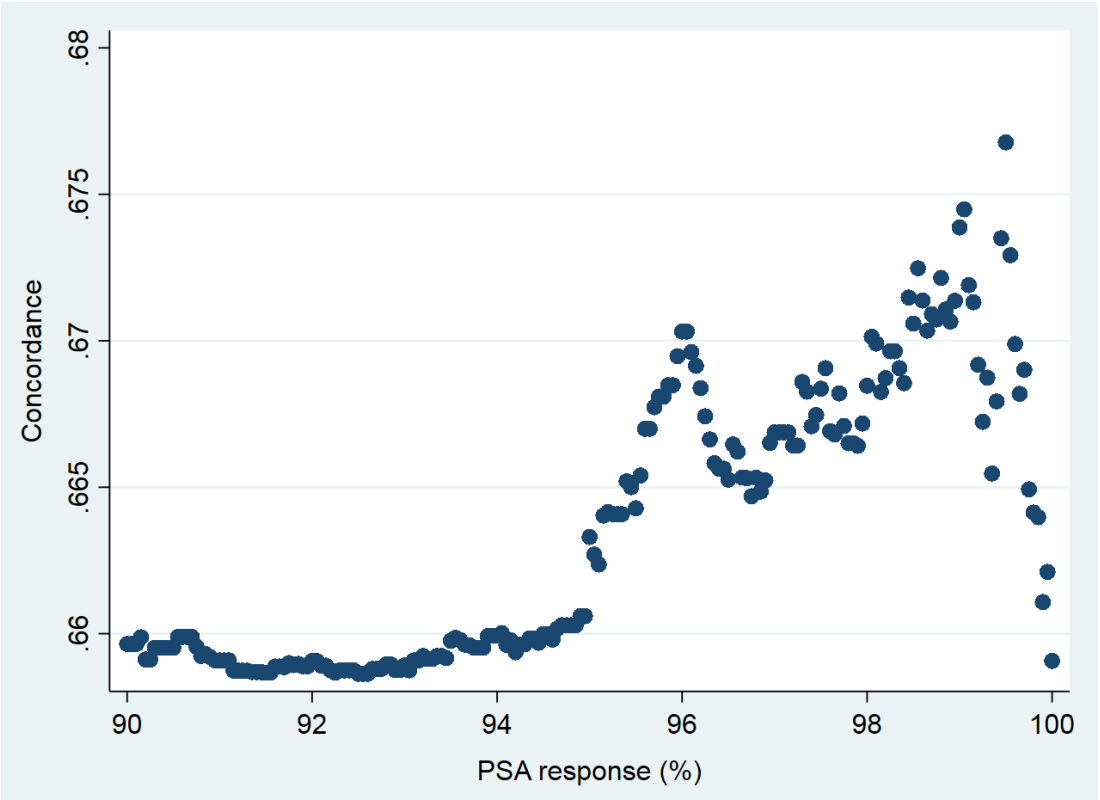
A baseline survival model was constructed and metastatic distribution, nodal stage, Gleason sum score, duration of prior ADT, week-6 PSA and performance stage at randomisation were all statistically significant at the 5% level, so included in the multivariable analysis, see **A4:** Table 69**.**

Threshold value

The observed relationship between PSA response and OS was different when compared with the ADT alone treated group. In a multivariable cox-regression model the concordance indices for a range of threshold PSA response values was consistently lower with the highest observed value around 67.5%, see **Graph 5**.

Taken together these results suggest the impact of docetaxel treatment had impacted PSA; the predictive discrimination value of this outcome is less, with no clear threshold value shown to have potential clinical utility. Therefore further analyses were not conducted.

Graph 5: PSA response threshold value in ADT+docetaxel group (concordance statistic)



Harroll's C-statistic was calculated for a range of threshold values. The highest predictive discriminatory value was only 67.5%, this is less than observed in the ADT alone treated group (78%) suggesting the prognostic impact of PSA response assessed at this time point is less in patients treated with ADT+docetaxel.

3.4 Patient cohort for analysis of PSA nadir assessed following completion of docetaxel

612 of the 727 (84%) metastatic ITT population allocated to receive docetaxel were eligible for inclusion in this analysis, see **Figure 25.** The most common reason for exclusion was not commencing docetaxel; 9% of metastatic patients did not report starting. Other reasons foe exclusion included a reported FFS event (i.e. PSA, symptomatic or radiological disease progression) prior to 24-weeks landmark. Only one additional case was excluded due to insufficient PSA data.

As shown in **Table 29,** the population excluded from the analysis had a slightly different metastatic distribution and more patients with a poor performance status, however otherwise the analysis population were comparable with the ITT population. All but one patient had completed docetaxel by 24-weeks post randomisation; 89% received ≥5 of the 6 planned cycles. Toxicity was the most frequent reason for early treatment stopping (71%).

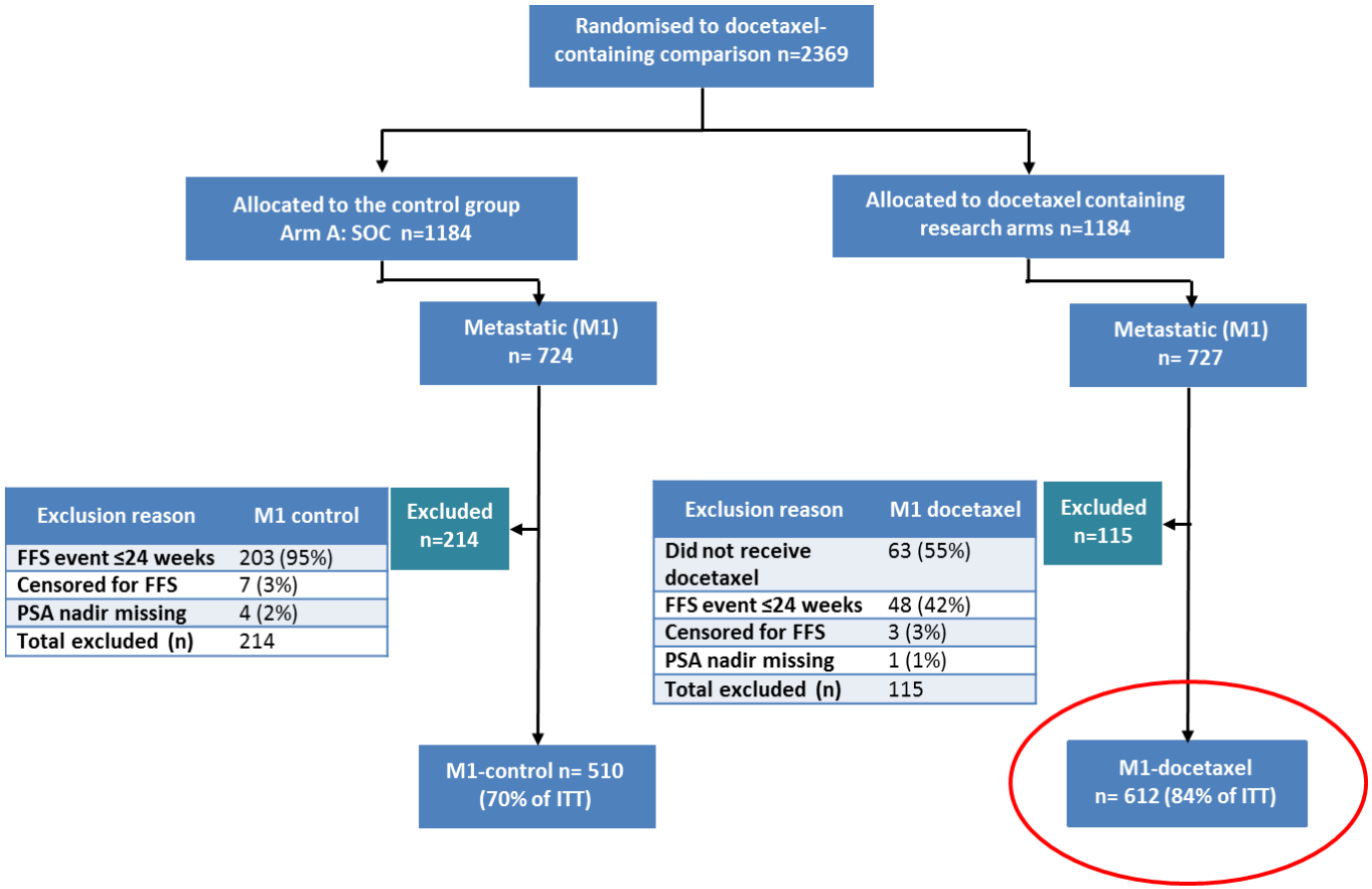
3.4.1 Comparative baseline characteristics by PSA nadir category

The clinical characteristics of the groups categorised by PSA nadir are shown in **Table 31**. PSA nadir >4ng/ml was associated with a higher presenting PSA, worse baseline performance status and a higher proportion of unknown Gleason score which may be explained by a histological diagnosis made from a metastatic sample.

3.4.2 PSA nadir

Within the docetaxel-treated population eligible for inclusion in analysis the median PSA nadir was 0.6ng/ml (IQR 0.2-2). In the comparable ADT alone treated population, median PSA nadir was 0.9ng/ml (IQR 0.3-2.9), see **Table 30** for distribution by category. The addition of docetaxel was associated with an increased proportion of patients achieving a PSA nadir of <0.2ng/ml (21% vs. 14%).

Figure 25: Patient selection for analysis of PSA nadir



The prognostic impact of PSA was explored in the patient group allocated to receive docetaxel who reported starting treatment and had not reported (or been censored) a failure-free survival event prior to the landmark. In total, 612 patients with metastatic disease (M1) at trial entry allocated to receive docetaxel met the inclusion criteria (84% of the ITT) as shown by the red circle.

Table 29: Comparative baseline characteristics in docetaxel treated cohort

Characteristic	Population		
	ITT (%)	Analysis	Excluded
	n=727	n=612	n=115
Tumour stage			
≤T2	97 (13%)	82 (13%)	15 (13%)
T3	391 (54%)	336 (55%)	55 (48%)
T4	165 (23%)	135 (22%)	30 (26%)
Tx	74 (10%)	59 (10%)	15 (13%)
Nodal stage			
N0	236 (33%)	207 (34%)	29 (25%)
N+	423 (58%)	347 (57%)	76 (66%)
Nx	68 (9%)	58 (9%)	10 (9%)
Metastatic distribution			
Bone only	447 (61%)	390 (64%)	57 (50%)
Distant nodal	79 (11%)	67 (11%)	12 (10%)
Bone & distant nodes, or other metastatic site	201 (28%)	155 (25%)	46 (40%)
Gleason sum score			
≤ 7	131 (18%)	114 (19%)	17 (15%)
≥ 8	499 (67%)	423 (69%)	76 (66%)
Unknown	97 (13%)	75 (12%)	22 (19%)
Age			
≤55	61 (8%)	53 (7%)	8 (7%)
55-59	98 (13%)	82 (13%)	16 (14%)
60-64	169 (23%)	138 (23%)	31 (27%)
65-69	192 (26%)	169 (28%)	23 (20%)
70-74	138 (19%)	116 (19%)	22 (19%)
≥ 75	69 (9%)	54 (9%)	15 (13%)
Performance status			
0	533 (73%)	453 (74%)	80 (70%)
1 or 2	194 (27%)	159 (26%)	35 (30%)
Presenting PSA (ng/ml)			
<18.6	108 (15%)	93 (15%)	15 (13%)
18.6 – 46.4	105 (14%)	90 (15%)	15 (13%)
46.5 – 94.2	143 (20%)	120 (20%)	23 (20%)
94.3 – 277.2	150 (21%)	124 (20%)	26 (23%)
>278	221 (30%)	185 (30%)	36 (31%)

Table 30: Frequency within PSA nadir categories

PSA category	Metastatic ITT	Analysis population	
		ADT+ docetaxel	ADT-alone
≤0.2ng/ml	210 (14%)	131 (21%)	71 (14%)
>0.2 - ≤4ng/ml	818 (56%)	385 (63%)	341 (67%)
>4mg	423 (29%)	96 (16%)	98 (19%)

Table 31: Comparative baseline characteristics by PSA nadir category

Baseline characteristic	PSA nadir ≤0.2ng/ml	PSA nadir >0.2 ≤4ng/ml	PSA nadir >4ng/ml	P value*
	n= 131 (21%)	**n=385 (63%)**	**n=96 (16%)**	
Tumour stage				
≤T2	16 (12%)	53 (14%)	13 (14%)	0.066
T3	71 (54%)	221 (57%)	44 (46%)	
T4	37 (28%)	72 (19%)	26 (27%)	
Tx	7 (5%)	39 (10%)	13 (14%)	
Nodal stage				
N0	42 (32%)	132 (34%)	33 (34%)	0.834
N+	79 (60%)	216 (56%)	52 (54%)	
Nx	10 (8%)	37 (10%)	11 (11%)	
Metastatic distribution				
Bone only	80 (61%)	249 (65%)	61 (64%)	0.171
Distant nodes only	19 (15%)	43 (11%)	5 (5%)	
Bone & nodes or other	32 (24%)	93 (24%)	30 (31%)	
Gleason sum score				
≤ 7	31 (24%)	72 (19%)	11 (11%)	**<0.001**
≥ 8	93 (71%)	271 (70%)	59 (61%)	
Unknown	7 (5%)	42 (11%)	26 (27%)	
Age				
≤55	11 (8%)	35 (9%)	7 (7%)	0.5752
55-59	14 (11%)	56 (15%)	12 (13%)	
60-64	27 (21%)	84 (22%)	27 (28%)	
65-69**	40 (31%)	105 (27%)	24 (25%)	
70-74	25 (19%)	72 (19%)	19 (20%)	
≥ 75	14 (11%)	33 (9%)	7 (7%)	
Performance status				
0	107 (82%)	281 (73%)	65 (68%)	**0.042**
1 or 2	24 (18%)	104 (27%)	31 (32%)	
Presenting PSA (ng/ml)				
≤20	48 (37%)	56 (15%)	1 (1%)	**0.0001**
21-50	27 (21%)	67 (17%)	3 (3%)	
51-100	21 (16%)	85 (22%)	12 (13%)	
101-250	18 (14%)	70 (18%)	10 (10%)	
>250	17 (31%)	107 (28%)	70 (73%)	
Duration of ADT prior to randomisation				
0 weeks***	4 (3%)	20 (5%)	5 (5%)	0.0666
≤2 weeks	23 (19%)	43 (11%)	9 (9%)	
≤4 weeks	19 (15%)	54 (14%)	8 (8%)	
≤6 weeks	23 (18%)	65 (17%)	16 (17%)	
≤8 weeks	24 (18%)	57 (15%)	24 (25%)	
≤10 weeks	18 (14%)	74 (19%)	12 (13%)	
≤12 weeks	17 (13%)	67 (17%)	22 (21%)	
≤14 weeks	1 (1%)	4 (1%)	0 (0%)	

*Kruskal-Wallis test of difference by group; statistically significant at 5% level shown in bold

** Contains median age

*** Started within 7 days post randomisation

3.4.3 Baseline survival model for analysis of PSA nadir

All baseline characteristics were evaluated in a multivariable model and those that remained significant at the 5% level included in the baseline survival model: metastatic distribution, Gleason sum score, baseline performance status and duration of prior ADT, see **Table 32.**

Table 32: Baseline multivariable survival model (PSA nadir)

Characteristic			Overall survival		
	Category description	**n**	**Events**	**Multivariable**	**p-value***
T stage	≤T2	82	29	0.90 (0.61-1.32)	
	T3	335	116	1.0	
	T4	135	55	1.10 (0.84-1.45)	
	Tx	59	29	1.24 (0.85-1.83)	0.9341
Nodal stage	N0	207	70	1.0	
	N+	346	131	1.45 (1.04-2.02)	
	Nx	58	28	1.38 (0.89-2.14)	0.0686
Metastatic distribution	Bone only	390	149	1.87 (1.07- 3.26)	
	Distant nodal only	66	14	1.0	
	Bone & nodal or other	155	66	2.17 (1.21-3.88)	**0.0191**
Gleason sum score	≤ 7	113	33	1.0	
	≥ 8	423	157	1.54 (1.06-2.25)	
	Unknown	75	39	1.81 (1.14-2.89)	**0.0286**
Age	≤55	53	20	0.85 (0.51-1.42)	
	55-59	82	35	1.13 (0.74-1.74)	
	60-64	138	49	0.86 (0.59-1.27)	
	65-69[a]	168	57	1.0	
	70-74	116	50	1.11 (0.75-1.64)	
	≥ 75	54	18	0.68 (0.40-1.17)	0.3929
Performance status	0	453	155	1.0	
	1 or 2	159	74	1.40 (1.06-1.85)	**0.0178**
Duration ADT prior to randomisation	0 week[b]	29	15	1.05 (1.02-1.09)	**0.0048**
	≤2 weeks	77	26		
	≤4 weeks	81	24		
	≤6 weeks	104	35		
	≤8 weeks	105	42		
	≤10 weeks	104	41		
	≤12 weeks	106	44		
	≤14 weeks	5	2		
Presenting PSA[c]	-	611	229	0.98 (0.90-1.07)	0.7111

[a] Median age 65yrs
[b] All patients who were yet to start ADT prior to randomisation, commenced within 7 days
[c] Natural Logarithmic transformation
*For variable in overall survival model, values statistically significant at 5% level shown in bold

3.4.4 Impact of PSA nadir on overall survival

Median survival from the 24-week landmark was significantly longer in patients who achieved a PSA nadir <0.2ng/ml, median survival 85 months (95% CI 59-NR) compared with those that did not; median survival 53 months (95% CI 49-60) if PSA nadir >0.2<4ng/ml, and 42 months (95% CI 34-64) if PSA nadir>4ng/ml. This difference was highly statistically significant in a multivariable analysis, see **Table 33** and **Graph 6.**

Graph 6: Differential survival by categorised PSA nadir in docetaxel treated cohort

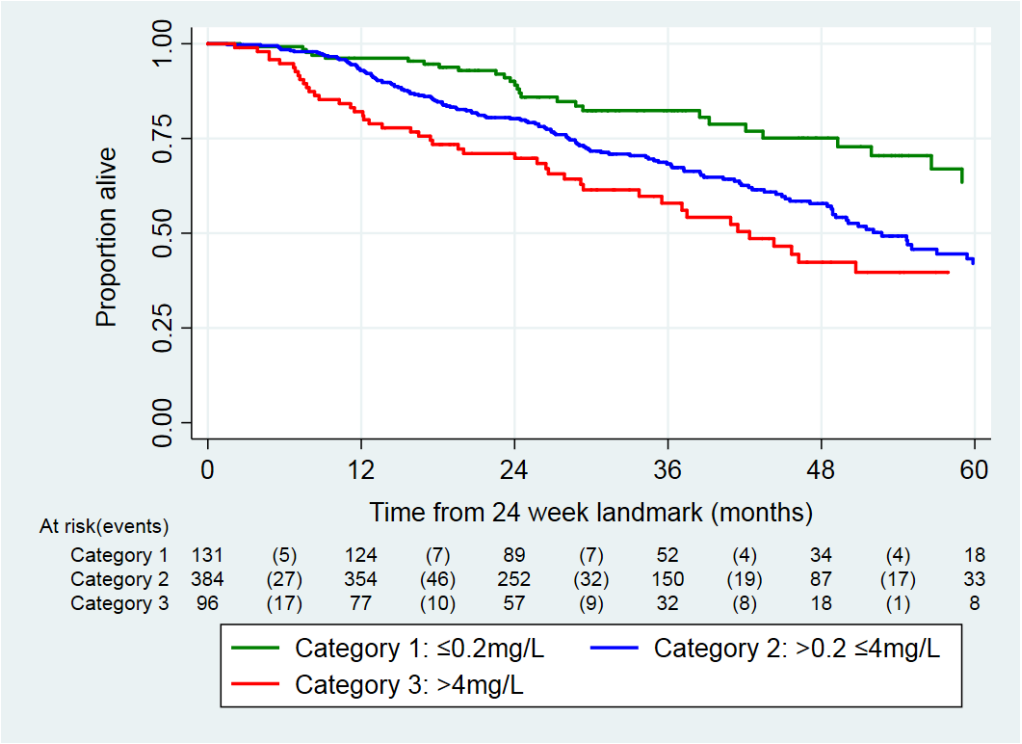


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Table 33: Summary of survival statistics (PSA nadir)

PSA nadir	n	Events	Median (95%CI)	3-yr OS	*Adjusted HR (95%CI)	P value
≤0.2ng/ml	131	29	85 months (59 – NR)	82%	1.0 (reference)	**0.0001**
>0.2 - 4ng/ml	384	154	53 months (49 – 60)	68%	1.99 (1.34-2.98)	
>4ng/ml	96	46	42 months (34 - 64)	58%	2.64 (1.64 – 4.26)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

3.4.5 Exploring additional threshold values

Three PSA thresholds previously shown to be prognostic when used as nadir and absolute values in ADT-treated cohorts were evaluated in this population, receiving the updated SOC ADT + docetaxel[180-182]. In addition, three further PSA threshold values shown to be prognostic in smaller ADT-treated cohorts were evaluated[187-189]. Thresholds of 1ng/ml and 2ng/ml were explored aiming to further differentiate within the 63% of the cohort who achieved a PSA nadir of between 0.2ng/ml and 4ng/ml. However neither of these values appeared to have additional prognostic significance, as shown by the overlapping survival curves **(Graph 7, Graph 8**). It was hypothesised that PSA nadir >10ng/ml may identify a very poor prognostic group, as has previously been seen in ADT-treated cohorts[187]. This threshold value identified a group with lower a 3 year survival rate (53%) however the effect was inconsistent over time and the estimated median survival time was the same as for the PSA nadir 4-10ng/ml category. Distinguishing if these groups have significantly different outcomes is limited by the relatively small numbers in each (n 49, 47), see **Graph 9.**

Graph 7: Differential survival by PSA nadir (threshold 1ng/ml)

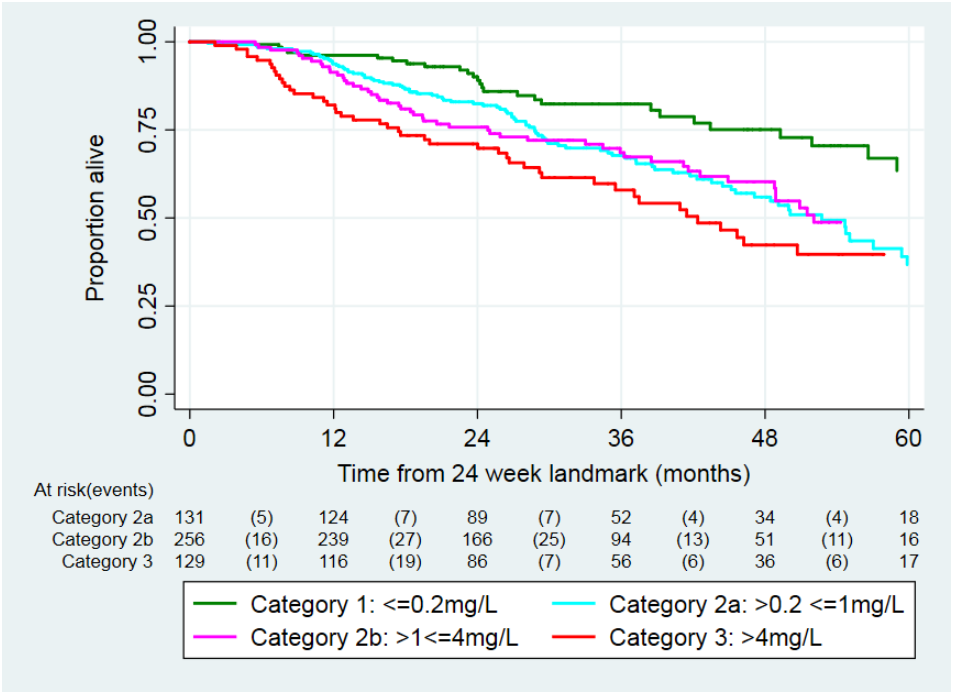


Table 34: Summary of survival by PSA nadir category (additional threshold 1ng/ml)

PSA nadir category	n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95%CI)	P value
≤0.2ng/ml	131	29	85 months (59 - NR)	82%	1.0 (reference)	**0.0003**
>0.2 to ≤1ng/ml	256	97	54 months (45 – 59)	68%	1.91 (1.25-2.91)	
>1 to ≤4ng/ml	129	57	53 months (45 - 68)	68%	2.15 (1.37 – 3.37)	
>4ng/ml	96	46	42 months (34-64)	58%	2.64 (1.64-4.26)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

Graph 8: Differential survival by PSA nadir (threshold 2ng/ml)

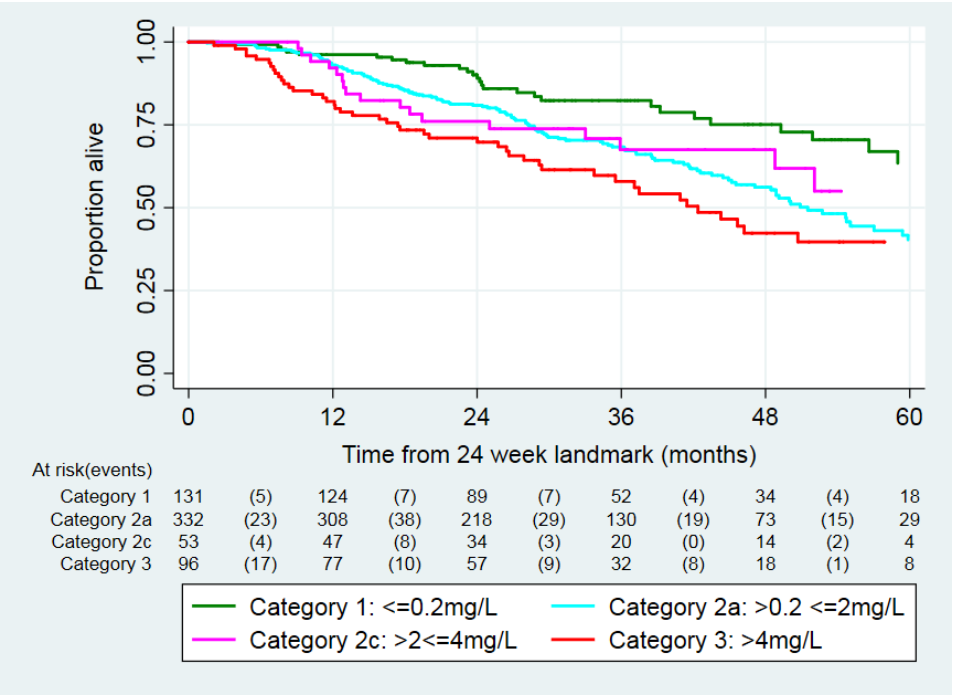


Table 35: Summary of survival by PSA nadir category (additional threshold 2ng/ml)

PSA nadir category	n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95%CI)	P value
≤0.2ng/ml	131	29	85 months (59 - NR)	82%	1.0 (reference)	**0.0003**
>0.2 to ≤2ng/ml	332	135	52 months (46 – 59)	68%	2.03 (1.36 - 3.05)	
>2 to ≤4ng/ml	52	19	70 months (49 - NR)	67%	1.76 (0.98 – 3.15)	
>4ng/ml	96	46	42 months (34 - 64)	58%	2.65 (1.64 – 4.27)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

Graph 9: Differential survival by PSA nadir (threshold >10ng/ml)

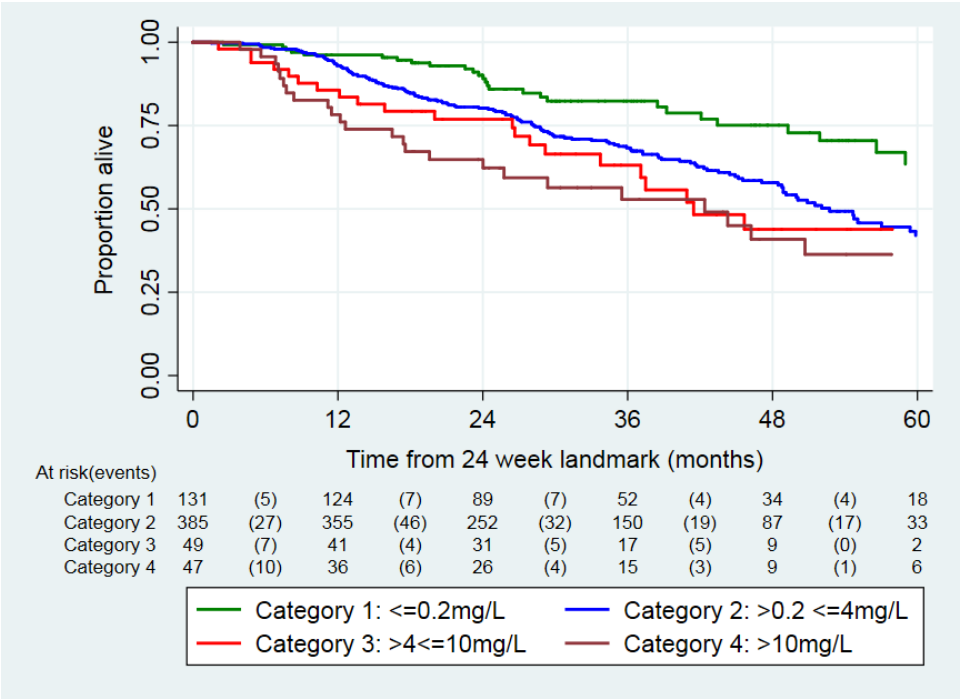


Table 36: Summary of survival by PSA nadir category (additional threshold >10ng/ml)

PSA nadir category	n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95%CI)	P value
≤0.2ng/ml	131	29	85 months (59 - NR)	82%	1.0 (reference)	**0.0002**
>0.2 to ≤4ng/ml	385	154	53 months (49 – 60)	68%	1.99 (1.33-2.97)	
>4 to ≤10ng/ml	49	21	41 months (34 - NR)	63%	2.31 (1.30 –4.11)	
>10ng/ml	47	25	42 months (20 - 64)	53%	3.01 (1.74-5.21)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

3.4.6 Relationship between PSA nadir and failure-free survival

As the STAMPEDE protocol definition of FFS is related to the PSA nadir value this outcome was considered less robust, as it may be biased and over-estimate the prognostic importance of a high PSA nadir. However FFS continues to have clinical value as an early intermediate outcome and therefore was explored as a secondary outcome, accepting this limitation. A baseline multivariable model was constructed. Baseline age, duration of ADT prior to randomisation and presenting PSA were all statistically significant and therefore included in the adjusted analysis. PSA nadir continued to be strongly prognostic in this model see **Table 37** and **Graph 10**. The baseline survival model is shown in **A5**: **Table 70**.

Graph 10: FFS by PSA nadir in docetaxel-treated cohort

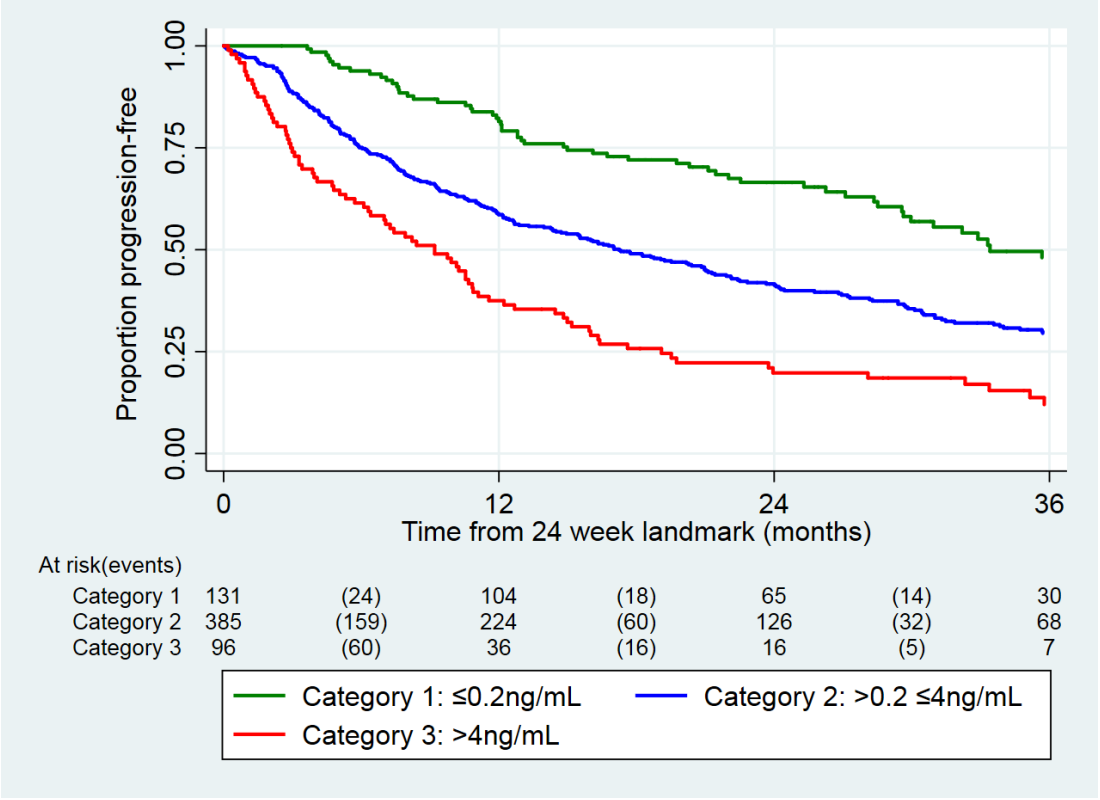


Table 37: FFS survival by PSA nadir category

PSA nadir	n	Events	Median (95%CI)	1-yr FFS *	**Adjusted HR (95%CI)	P value
≤0.2ng/ml	131	64	33 months (30 – 50)	81%	1.0 (reference)	<**0.0001**
>0.2- 4ng/ml	385	272	17 months (14 – 21)	59%	1.83 (1.38-2.41)	
>4ng/ml	96	83	9 months (6 - 11)	38%	2.97 (2.07 – 4.24)	

* Timed from landmark (24-weeks post randomisation)
**Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

3.4.7 Relationship between PSA nadir and survival in ADT alone group

A secondary analysis of PSA nadir and overall survival was performed in the STAMPEDE control group who received ADT alone until progression to CRPC (n=510, see **Figure 25**). This allows comparison with other published analyses that have explored PSA nadir and absolute PSA decline in ADT alone treated groups[180,181,187,190]. The median duration of ADT prior to the landmark was 30 weeks (IQR 27-33). At the 24 week landmark, 27% of patients allocated to the control group had already reported a FFS event. These patients were excluded because if second-line treatment had been started for progressive disease the nadir value may no longer be reflective of response to ADT alone. Additionally, these patients no longer fulfilled the definition of CSPC. Consistent with an aggressive clinical course, the excluded population had a higher disease burden, with more widespread metastatic distribution, higher tumour stage and a higher likelihood of pelvic nodal involvement, see **Table 38.**

In the population who were progression-free at 24-weeks post randomisation (around 30 weeks post ADT initiation) there is no good evidence that PSA nadir was associated with overall survival, see **Graph 11**. Although a similar trend was observed, in a multivariable model adjusted for metastatic distribution, Gleason score, baseline age and performance status, PSA nadir did not remain statistically significant (p 0.1462), see **Table 40.**

Table 38: Baseline characteristics (ADT alone treated population)

Baseline characteristic	Metastatic ITT	Eligible for PSA nadir analysis	Excluded
	n=724	**n=510**	**n=214**
Tumour stage			
≤T2	90 (12%)	70 (14%)	20 (9%)
T3	404 (56%)	297 (58%)	107 (50%)
T4	163 (23%)	106 (21%)	57 (27%)
Tx	67 (9%)	37 (7%)	30 (14%)
Nodal stage			
N0	242 (33%)	170 (33%)	72 (34%)
N+	416 (57%)	293 (57%)	123 (57%)
Nx	66 (9%)	47 (9%)	19 (9%)
Metastatic distribution			
Bone only	454 (63%)	319 (63%)	135 63%)
Distant nodes only	63 (9%)	54 (11%)	9 (4%)
Bone & nodes or other	207 (29%)	137 (27%)	70 (33%)
Gleason sum score			
≤ 7	156 (22%)	119 (23%)	37 (17%)
≥ 8	476 (66%)	335 (66%)	141 (66%)
Unknown	92 (13%)	56 (11%)	36 (17%)
Age			
≤55	70 (10%)	39 (8%)	31 (14%)
55-59	104 (14%)	70 (14%)	34 (16%)
60-64	146 (20%)	100 (20%)	46 (22%)
65-69[a]	187 (26%)	141 (28%)	46 (22%)
70-74	134 (19%)	100 (20%)	34 (16%)
≥ 75	83 (11%)	60 (12%)	23 (11%)
Performance status			
0	521 (72%)	391 (77%)	130 (61%)
1 or 2	203 (28%)	119 (23%)	84 (39%)
Presenting PSA (ng/ml)			
≤20	125 (17%)	101 (20%)	23 (11%)
21-50	121 (17%)	94 (18%)	27 (13%)
51-100	114 (16%)	84 (16%)	30 (14%)
101-250	143 (20%)	104 (20%)	39 (18%)
>250	221 (31%)	126 (25%)	95 (44%)
Duration of ADT prior to randomisation			
0[b]	44 (6%)	37 (7%)	7 (2%)
≤2 weeks	88 (12%)	65 (13%)	23 (11%)
≤4 weeks	108 (12%)	80 (16%)	28 (13%)
≤6 weeks	109 (33%)	68 (13%)	41 (19%)
≤8 weeks	129 (18%)	91 (18%)	38 (18%)
≤10 weeks	114 (16%)	84 (16%)	30 (14%)
≤12 weeks	125 (17%)	80 (16%)	45 (21%)
≤14 weeks	7 (1%)	5 (1%)	3 (1%)

[a] Median age 65yrs,

[b] All patients who were yet to start ADT prior to randomisation, commenced within 7 days

Table 39: Multivariable baseline survival model (ADT alone treated population)

Characteristic			Overall survival		
	Category description	n	Events	Multivariable	p-value*
T stage	≤T2	80	20	0.78 (0.48-1.28)	
	T3	297	113	1.0	
	T4	106	41	1.06 (0.74-1.53)	
	Tx	37	18	1.21 (0.72-2.04)	0.6003
Nodal stage	N0	170	58	1.0	
	N+	293	114	1.14 (0.79-1.64)	
	Nx	47	20	1..06 (0.63-1.80)	0.7760
Metastatic distribution	Bone only	319	128	2.97 (1.55-5.68)	
	Distant nodal only	54	10	1.0	**0.0005**
	Bone & nodal or other	137	54	2.97 (1.49-5.90)	
Gleason sum score	≤ 7	119	31	1.0	
	≥ 8	335	139	1.95 (1.31-2.90)	
	Unknown	56	22	1.53 (0.88-2.67)	**0.0010**
Age	≤55	39	23	2.58 (1.54-4.31)	
	55-59	70	33	1.73 (1.10-2.73)	
	60-64	100	40	1.27 (0.83-1.95)	
	65-69[a]	141	44	1.0	
	70-74	100	31	1.06 (0.67-1.68)	
	≥ 75	60	21	1.21 (0.72-2.05)	**0.0027**
Performance status	0	391	138	1.0	
	1 or 2	119	54	1.53 (1.11-2.10)	**0.0110**
Duration ADT prior to randomisation	0 week[b]	37	14	0.99 (0.95-1.03)	0.5508
	≤2 weeks	65	25		
	≤4 weeks	80	37		
	≤6 weeks	68	29		
	≤8 weeks	91	26		
	≤10 weeks	84	31		
	≤12 weeks	80	28		
	≤14 weeks	5	2		
Ln presenting PSA[c]	-			0.97 (0.88-1.06)	0.4411

[a] Median age 65yrs

[b] All patients who were yet to start ADT prior to randomisation, commenced within 7 days

[c] Natural Logarithmic transformation

*For variable in overall survival model, values that are statistically significant at 5% level shown in bold

Graph 11: Differential survival by PSA nadir in patients receiving ADT alone

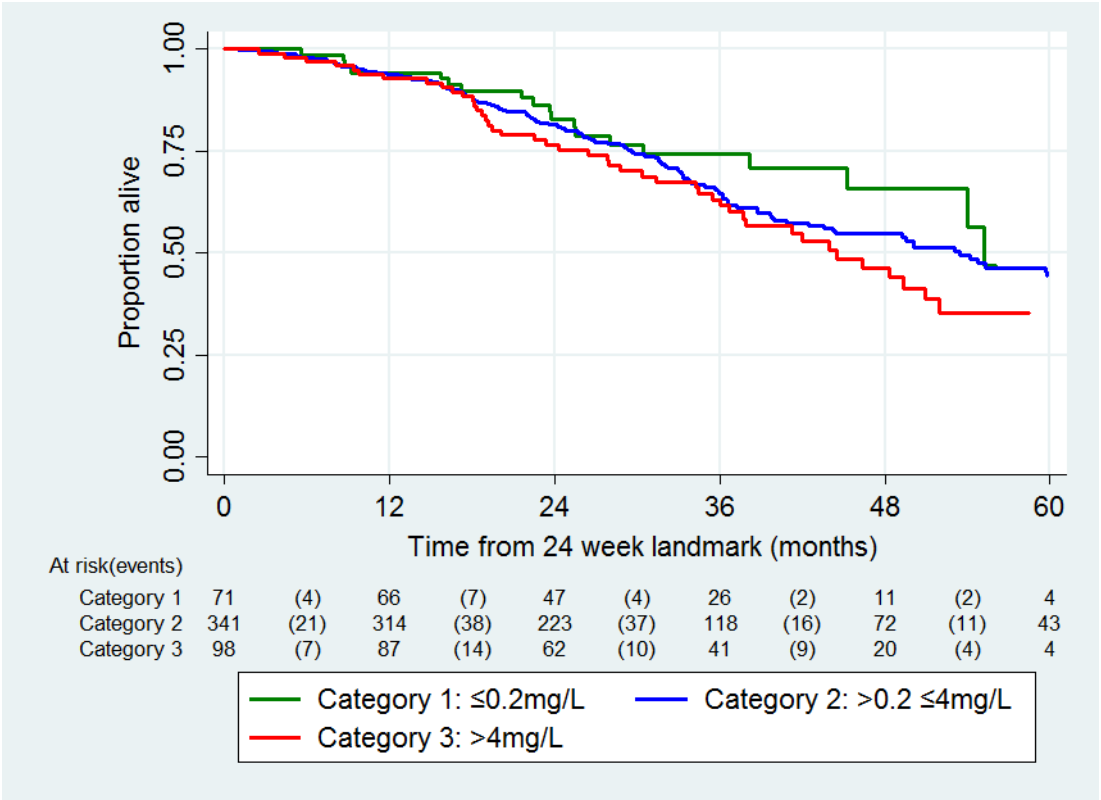


Table 40: Summary of survival by PSA nadir in patients receiving ADT alone

PSA nadir category	n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95%CI)	P value
≤0.2ng/ml	71	19	55 months (45- NR)	74%	1.0 (reference)	0.1462
>0.2 to ≤4ng/ml	341	129	53 months (42 – 63)	65%	1.14 (0.70-1.87)	
>4ng/ml	98	44	45 months (37 - 52)	63%	1.59 (0.92-2.75)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

Comparative survival analyses

These results do not show PSA nadir assessed around 30 weeks after commencing ADT is prognostic of OS. This contrasts with the findings of other similar analysis conducted within the SWOG 9346 and CHAARTED trial cohorts. As reported by Hussain *et al*. and Harshman *et al*. respectively, these data would suggest that when the same PSA threshold values (<0.2ng/ml, 0.2-4ng/ml, >4ng/ml) are assessed as absolute values at a landmark, they are prognostic of clinically significant survival differences in men with mCRPC treated with ADT +/- docetaxel[180,181].

However, the approach adopted by these investigators differs in two ways, the population and the definition of the PSA outcome. Firstly, all participants were included regardless of whether disease progression had occurred by the landmark assessment of PSA. Secondly, the PSA outcome is an absolute value assessed at a single point in time. In order to contextualise these data, I undertook a further exploratory analysis to assess the impact of defining the population differently, specifically including the subgroup experiencing early progressive disease.

To highlight the impact of this differing approach, **Graph 13** demonstrates the relative prognosis of the group in whom disease progression was reported within the first 24 weeks post randomisation (around 30 weeks after commencing ADT). As summarised in **Table 42**, these results demonstrate that this group have a distinct and very poor prognosis; median survival from the 24 week landmark was 17 months (95% CI 14- 19 months). This is equivalent to a median survival time of 2 years from the point of starting ADT. In an adjusted multivariable analysis this group were at 3 times greater risk of death compared with the group with a PSA nadir >4ng/ml but who reached the 24 week landmark progression-free; HR 3.10 (95% CI 2.18-4.40); p<0.0001. As shown in **Table 43**, progression prior to 24 weeks was associated with younger age, higher disease burden, higher presenting PSA and a poorer baseline performance status.

Graph 12 and **Table 41** summarise the results where all participants are categorised based on the recorded nadir value, but without excluding those in whom an FFS event (e.g. PSA progression) has been reported prior to the landmark. In keeping with the approach adopted in the analyses of the CHAARTED and SWOG 9346 cohorts, all patients experiencing disease progression were grouped in the PSA nadir >4ng/ml category[180,181].

Graph 12: OS by PSA nadir including patients experiencing early progression (ADT alone)

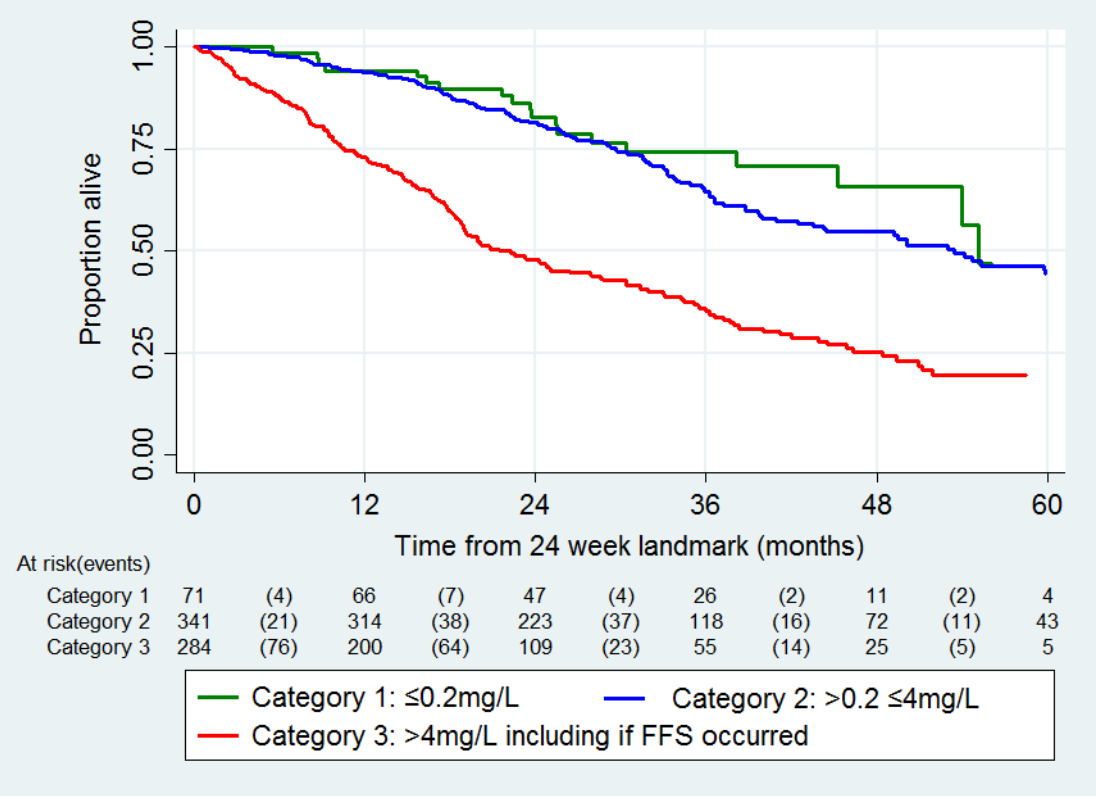


Table 41: Summary of OS including patients experiencing early progression (ADT alone)

PSA nadir category	n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95% CI)	P value
≤0.2ng/ml	71	19	55 months (45 - NR)	74%	0.30 (0.19-0.49)	**<0.0001**
>0.2 to ≤4ng/ml	341	129	53 months (42 – 63)	65%	0.36 (0.29-0.46)	
>4ng/ml	301	199	21 months (19 - 28)	36%	1.0 (reference)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

Graph 13: OS by PSA nadir highlighting distinct clinical course of patients experiencing early progression (ADT alone)

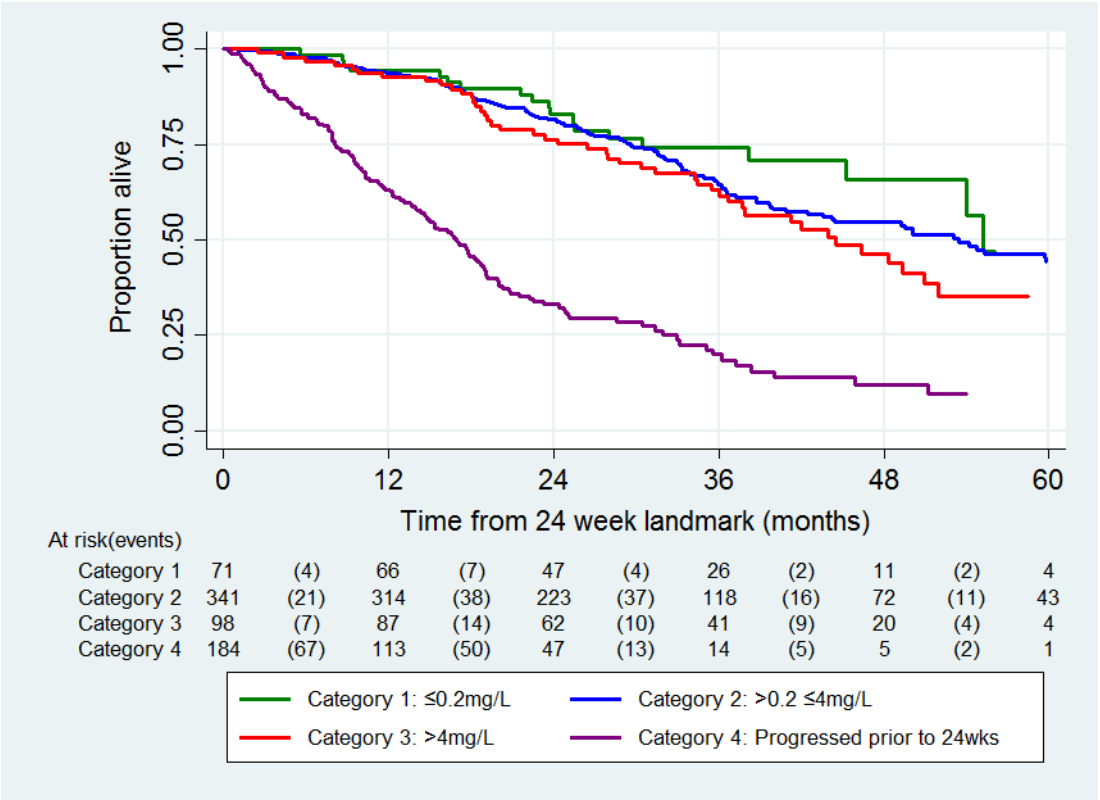


Table 42: Summary survival statistics including patients experiencing early progression

Category		n	Events	Median (95%CI)	3 year OS	*Adjusted HR (95%CI)	P value
Disease progression reported prior to 24 week landmark		198	150	17 months (14-19)	20%	3.10 (2.18-4.40)	**<0.0001**
PSA nadir category	**≤0.2ng/ml**	72	20	55 months (45 - NR)	74%	0.63 (0.36-1.08)	
	>0.2 to 4ng/ml	342	129	53 months (42 – 63)	65%	0.75 (0.29-0.46)	
	>4ng/ml	99	45	45 months (37 - 52)	63%	1.0 (reference)	

*Adjusted for metastatic distribution, Gleason score, baseline performance status and duration of prior ADT

Table 43: Comparative baseline characteristics (including progression <24 weeks)

	PSA nadir ≤0.2ng/ml	PSA nadir >0.2 ≤4ng/ml	PSA nadir >4ng/ml	Progressed prior to 24 weeks	P value*
	n=72 (10%)	**n=342 (48%)**	**n=99 (14%)**	**n=198 (28%)**	
Tumour stage					
≤T2	14 (19%)	47 (14%)	11 (11%)	16 (8%)	
T3	40 (56%)	201 (59%)	56 (57%)	103 (52%)	
T4	12 (17%)	73 (21%)	22 (22%)	52 (26%)	
Tx	6 (8%)	21 (6%)	10 (10%)	27 (14%)	
Nodal stage					
N0	28 (39%)	117 (34%)	26 (26%)	70 (35%)	0.542
N+	39 (54%)	196 (57%)	60 (61%)	111 (56%)	
Nx	5 (7%)	29 (8%)	13 (13%)	17 (9%)	
Metastatic distribution					
Bone only	47 (65%)	218 (64%)	55 (56%)	128 (65%)	**0.015**
Distant nodes only	8 (11%)	35 (10%)	11 (11%)	6 (3%)	
Bone & nodes or other	17 (24%)	89 (26%)	33 (33%)	64 (32%)	
Gleason sum score					
≤ 7	21 (29%)	78 (23%)	21 (21%)	33 (17%)	**0.013**
≥ 8	44 (61%)	234 (68%)	59 (60%)	132 (67%)	
Unknown	7 (10%)	30 (9%)	19 (19%)	33 (17%)	
Age					
≤55	3 (4%)	26 (8%)	10 (10%)	29 (15%)	**0.0028**
55-59	3 (4%)	50 (15%)	17 (17%)	32 (16%)	
60-64	13 (18%)	74 (22%)	13 (13%)	43 (22%)	
65-69 [a]	26 (36%)*	87 (25%)*	29 (29%)*	43 (22%)	
70-74	16 (22%)	65 (19%)	21 (21%)	29 (15%)	
≥ 75	11 (15%)	40 (12%)	9 (9%)	22 (11%)	
Performance status					
0	55 (76%)	265 (77%)	73 (74%)	123 (62%)	**0.002**
1 or 2	17 (24%)	77 (23%)	26 (26%)	75 (38%)	
Presenting PSA (ng/ml)					
≤20ng/ml	30 (42%)	71 (21%)	1 (1%)	20 (10%)	**0.0001**
21-50ng/ml	17 (24%)	75 (22%)	3 (3%)	23 (12%)	
51-100ng/ml	7 (10%)	65 (19%)	12 (13%)	30 (15%)	
101-250ng/ml	5 (7%)	66 (19%)	33 (33%)	38 (19%)	
>250ng/ml	13 (18%)	65 (19%)	50 (51%)	87 (44%)	
Duration of ADT prior to randomisation					
0 [b]	5 (7%)	28 (8%)	4 (4%)	6 (3%)	0.067
≤2 weeks	10 (14%)	41 (12%)	14 (14%)	21 (11%)	
≤4 weeks	12 (17%)	53 (16%)	17 (17%)	24 (12%)	
≤6 weeks	6 (8%)	49 (14%)	13 (13%)	39 (20%)	
≤8 weeks	14 (19%)	59 (17%)	18 (18%)	36 (18%)	
≤10 weeks	12 (17%)	57 (17%)	15 (15%)	29 (15%)	
≤12 weeks	11 (15%)	53 (16%)	17 (17%)	42 (21%)	
≤14 weeks	2 (3%)	2 (1%)	1 (1%)	1 (1%)	

[a] Median age 65yrs,

[b] All patients who were yet to start ADT prior to randomisation, commenced within 7 days

*Kruskal Wallis test for difference, values statistically significant at 5% level shown in bold

3.5 Discussion

The addition of docetaxel chemotherapy to ADT is now recommended for all men presenting with metastatic castrate-sensitive prostate cancer who are judged fit enough. However in the predominantly elderly co-morbid population affected by PCa this can be a complex assessment of the relative risks and benefits[24,191]. Emerging data describing "real-world" use of docetaxel suggests whilst similar PFS gains are observed, toxicity is increased compared with that observed in trial populations. In particular, a higher incidence of febrile neutropenia is observed; 17-20% compared with 12% reported by the STAMPEDE trial[28,29]. In a recently reported small non-trial cohort of 103 patients receiving docetaxel for mCSPC, 34% were hospitalised and 2% died within 30 days of receiving chemotherapy[28]. The risk of febrile neutropenia is suggested to be inversely related to time between starting ADT and docetaxel; the OR of febrile neutropenia is 9.75 (95% CI 1.2-77.7; p 0.032) if docetaxel is started ≤19 days after starting ADT, but if 60-79 days have elapsed the OR is reduced to 1.86 (0.28-12.16). Although the number of events are small, these preliminary data suggest that a strategy of initiating ADT and waiting to assessing PSA response before deciding on docetaxel use may be beneficial. Through an additional analysis of the metastatic subgroup within the STAMPEDE control arm, I sought to test the hypothesis that the magnitude of PSA response assessed around 12 weeks (IQR 9.1-15.0) after commencing ADT would be prognostic of OS and therefore improve risk-stratification in this disease setting.

PSA responses to ADT were large (median 97%; IQR 91-95%) and 95% experienced a response of >50%. This is comparable with previous cohorts of newly diagnosed metastatic castrate-resistant disease; in a retrospective review of 982 patients 91% were reported to experience a ≥50% PSA response to ADT alone[192]. This consistently high response rate contrasts with those observed in mCRPC cohorts, where PSA responses of ≥30% and ≥50% are frequently reported and may be included in composite assessments of benefit in the castrate-resistant setting. The PCWG classifies PSA response as an "activity estimating endpoint" but has not recommended thresholds specific to the castrate-sensitive setting. This may simply reflect that, until recently, this patient group uniformly received ADT alone, meaning there was little clinical need to assess activity[169].

The magnitude of PSA response was shown to associate with improved OS in patients receiving ADT alone ($p<0.0001$). To be applicable to clinical practice, prognostic categories must be defined. As there were no established thresholds I sought to define these within this cohort. A PSA response of ≥99% was found to have the greatest predictive

discrimination of differences in OS. 3 year survival was 73% compared with 61% if the PSA response was 80-99%, equivalent to a 13 month difference in median OS times. However, in a multivariable analysis, the statistical significance of PSA response was reduced in the model that included absolute PSA value at the landmark, suggesting with both the relative and absolute PSA reduction may be significant.

PSA responses of <80% were fortunately rare occurring in 13% but were associated with significantly worse survival outcome; median survival of 21 months compared with 45 months if a PSA response of 80-98% was observed. In the multivariable analysis, a PSA response of <80% was associated with almost a 3-fold increase in the risk of death (HR 2.94; 2.20-3.93, p<0.0001). Again, data acquired in mCRPC cohorts supports this trend although the magnitude of difference is smaller, consistent with the hypothesis that the prognostic effect of PSA is greatest in the castrate-sensitive setting, when AR-responsive PSA transcription would be most reflective of therapeutic response[173,174]. Together these data suggest that PSA response assessed around 12 weeks post initiation of ADT in men with mCSPC can provide additional prognostic information that may be clinically useful when considering the use of first-line docetaxel.

Strengths of this analysis include the availability of prospectively collected PSA data for 93% of the STAMPEDE cohort, thus avoiding the bias that retrospective analyses may risk through systematic differences in those with and without available PSA data. Other strengths include the completeness of the data and length of follow-up. However, the median age of this cohort was 65 years (at least 10 years younger than the average clinic population) and all patients recruited to STAMPEDE during this period had to be considered fit for chemotherapy. Therefore, whilst prognostic factors can be explored within this cohort, external validation in groups more representative of clinic populations would be important in confirming their utility in risk-stratification and informing the decision as to whether to give docetaxel. Other ongoing trials in this setting such as PATCH (NCT00303784) may be useful sources of external validation. The population enrolled in PATCH to date have been older and fewer patients are receiving SOC docetaxel (~50% compared with ~90% in STAMPEDE) suggesting that this is more typical of a population in whom additional risk-stratification could be useful.

A limitation of this analysis is that the timing of PSA data collection does not allow us to explore the interaction of PSA response with subsequent docetaxel use, as PSA at randomisation is not collected within the STAMPEDE protocol, so we lack comparative

baseline PSA data. The median observed survival time in the group who experienced a PSA response of ≥99% following ADT alone is 58 months (IQR 46-66), which is closer to the estimated median observed for the docetaxel treated metastatic subgroup than it is the ADT alone metastatic subgroup[20]. However, what is unclear is where the groups defined by PSA response lie in the wide variation in survival times observed for both treatment strategies. Within the randomised docetaxel comparison the median survival observed was 45 months (IQR 23-91) for mCSPC treated with ADT alone, and 60 months (IQR 27-103) in the group allocated to receive ADT + docetaxel. Analysis of comparable pre-treatment PSA data within a randomised cohort is required to determine whether the magnitude of benefit for docetaxel differs within the risk categories identified in this analysis. This information would be required to support not using docetaxel in selected patients with a PSA response ≥99%, as although the results of the analysis show this group to have a comparatively good outcome relative to those achieving smaller PSA responses, the potential survival gain of additional docetaxel is not known.

The second objective of this analysis was to assess whether PSA nadir assessed following completion of SOC docetaxel is prognostic of survival outcome. This second on treatment biomarker is proposed to have potential clinical utility in identifying a subgroup who remain at high-risk of death despite docetaxel, in whom additional intensified treatment strategies could be evaluated. Results showed that the failure to achieve a PSA nadir of <4ng/ml was associated with significantly worse survival outcome, with 3 year survival rates of 58% compared with 82% if PSA nadir reached <0.2ng/ml; HR 2.64 (95% CI 1.64-4.26) p<0.0001. 21% of patients treated with ADT + docetaxel achieved a PSA nadir <0.2ng/ml and docetaxel use increased the likelihood of this. Recently reported results from the CHAARTED trial[180] also demonstrate that docetaxel use is associated with improved PSA response, however the approach to this analysis differs in the PSA measurement, assessment timing and the patient population, see **Table 44.**

Table 44: Comparison of main trial cohorts in which PSA has been evaluated

	SWOG 9346	CHAARTED	STAMPEDE
Population	• n=1345 • Treatment: ADT alone	• n=719 • Randomised to receive ADT + docetaxel or ADT alone	• n=612 • Treatment: ADT + docetaxel
Inclusion criteria	• PSA ≥5ng/ml	• ≥7 months FU after ADT initiation • 7-month post ADT PSA	• Sufficient PSA data • No FFS event prior to landmark
PSA measurement	**Absolute PSA** level at 6 and 7 months post registration	**Absolute PSA** level 7 months post initiation of ADT	**PSA nadir** lowest PSA reported at any time point within first 24 weeks post randomisation
ADT prior to assessment of PSA nadir	Maximum 13 months • Late registration group (~30%) received ≤6 months pre-registration • All received 7 months post registration	Maximum 7 months • ADT could start ≥4 months prior to randomisation but landmark set 7 months post initiation of ADT regardless of when this started	Maximum 9 months • ADT can start ≥12 weeks prior to randomisation • PSA assessed 24 weeks post randomisation

The PSA measurement assessed in the STAMPEDE cohort was PSA nadir, defined as the lowest PSA reported in the first 24 weeks on trial. Given that up to 12 weeks ADT is permitted prior to randomisation, this equates to an assessment of response that may occur from randomisation up until 36 weeks/8 months after starting ADT; the median time point in this cohort was around 30 weeks. This landmark corresponds to when docetaxel has been completed, typically within the previous 6 weeks. In contrast, the approach adopted in the CHAARTED analysis was a snapshot assessment of PSA 7-months post starting ADT. This absolute PSA value measured at one point in time has the advantage that it is easily replicated outside of trial setting; however it is unclear whether docetaxel was complete and therefore whether this PSA value is reflective of full response. Data in ADT alone treated cohorts have suggested the median time to nadir is 8-9 months[183,187,190] so these measurements are unlikely to be directly comparable. When both absolute 6-month PSA and PSA nadir were assessed in a cohort of 286 men with mCSPC, only PSA nadir remained significant on the multivariable analysis, suggesting that, as is seen with measures such as PSA doubling time, kinetic measures of PSA may be more informative than assessments made at a single point in time[166,190].

A further difference between my approach to this analysis and that adopted within the CHAARTED cohort is how the eligible population is defined. One of the strengths of the STAMPEDE cohort is its large size that permits investigation of PSA nadir in the group receiving ADT and docetaxel (n=612). In addition, only those who reached the landmark progression-free, such that they fulfilled the definition of CSPC were included. This allowed me to address the question of whether PSA nadir could be used as a prognostic biomarker in docetaxel-treated patients capable of identifying a population in whom to evaluate intensified CSPC treatment. The rationale being that patients who experience disease progression prior to this landmark have declared themselves as having poor prognostic disease and will likely commence treatment for CRPC and therefore have less need for further risk-stratification. In contrast, CHAARTED randomised a total of 790 patients and grouped both ADT and ADT + docetaxel treated patients together for the analysis of PSA response, stratifying for treatment allocation. All patients were categorised according to the absolute 7-month PSA measurement and patients experiencing prior progression were included in >4ng/ml group. Therefore, the CHAARTED population may be considered more heterogeneous, containing patients fulfilling the definition of castrate-sensitive, but also castrate-resistant disease and who have received different first treatments. Significantly, the PSA >4ng/mL group was used as the reference group in the multivariable analysis (i.e. the group that contains early patients with early CRPC) likely increasing the differences observed in prognosis.

Within my analysis, the results of the group allocated to receive ADT alone highlight that progression within around 30 weeks (range 24-36) of starting ADT is associated with a distinctly poor prognosis. A failure-free survival time of <24 weeks was observed in 28% patients receiving ADT alone and was associated with a very poor outcome; 3 year survival rate of 20% and median survival 17 months (95% CI 14-19), considerably worse than the group overall (45 months). This group was characterised as younger, with a high disease burden, higher presenting PSA and worse baseline performance status. The proportion of early treatment failures on ADT is considerable higher in STAMPEDE compared with the CHAARTED or SWOG 9346 cohorts[180,181]. This may be explained by the longer duration of ADT prior to the landmark analysis in STAMPEDE, together with the likelihood that the other trials selected patients with an improved response to ADT. In contrast to STAMPEDE, the CHAARTED protocol explicitly excluded patients showing signs of progression, whilst the SWOG 9346 induction phase specifically aimed to identify ADT-responsive cases suitable for randomisation to intermittent therapy[180,181].

Although the methods differ, the results of this analysis and that conducted within the CHAARTED trial cohort both demonstrate that patients receiving ADT + docetaxel who experience poor PSA response, either defined by PSA nadir or absolute value assessed 7-months after starting ADT, are at high-risk of early death. These data support the need to evaluate additional treatment strategies in these groups. Flaig *et al.* recently reported a small phase 2 study which recruited 41 patients who had a poor response to ADT, defined as a 7-month PSA >4ng/ml and evaluated whether the addition of abiraterone increased the proportion of patients subsequently achieving a PSA level <0.2ng/ml within 12 months. Although the pre-specified threshold of 20% (requiring 6 patients of the target 38 to achieve primary outcome) was not met, 5 patient (12%) did subsequently achieve a PSA <0.2ng/ml and a further 8 (20%) achieve a PSA <4ng/ml[193].

3.5.1 Future work

Future validation of these data is possible within the STAMPEDE dataset. The PSA response finding in ADT treated patients may be validated within participants allocated to any of the trial arms where an improvement in FFS or OS has not been observed e.g. non-overlapping controls to other comparisons, or those allocated to the celecoxib or zoledronic acid single agent arms. However, validation using other clinical trial datasets is challenging whilst PSA based outcomes are non-standardised and therefore protocol specific. Based on these data, the STAMPEDE protocol has been revised (version 19.0) to include collection of PSA at randomisation. This will permit analyses of PSA response (albeit at a slightly earlier time point) in randomised cohorts including testing for treatment interaction to address whether treatment effect varies within each prognostic risk category, for example through stratified randomisation and sub-group analyses. PSA nadir continues to be collected for all STAMPEDE participants and therefore subsequent analyses could explore if this is prognostic in groups receiving alternative therapies e.g. abiraterone. The prior hypothesis would be that as abiraterone is an AR-targeted therapy, PSA is more likely to capture treatment effect and therefore the prognostic impact may be even greater, such that it may be appropriate to asses surrogacy if prognostic effect is confirmed. Taken together, these data would support the further examination of PSA based outcomes as pragmatic, readily available biomarkers that may be able to inform treatment decisions if prognostic effect is validated.

3.6 Conclusions

Both the magnitude of PSA response and PSA nadir are shown to associate with survival differences within the STAMPEDE cohort. PSA response assessed around 12 weeks after ADT initiation was shown to associate with survival differences, suggesting that this could be an early indicator of risk of death, clinically useful in considering whether to intensify treatment to include docetaxel or abiraterone in addition to ADT. Alone, the discriminatory value of the biomarker is reduced in a survival model adjusted for absolute PSA value at the landmark. Future evaluation in a larger cohort would enable this potential interaction to be explored further. PSA nadir, previously shown to be prognostic of survival in patients within the STAMPEDE control arm receiving ADT-alone, was shown to remain so in patients receiving ADT + docetaxel, the updated SOC[182]. These data would support further investigation and prospective validation of both these on treatment biomarkers.

Chapter 4 Genomic profiling in metastatic castrate-sensitive prostate cancer: a feasibility and prevalence study

4.1 Introduction

4.1.1 Background

Due to recent technological advances it is now possible to genetically profile cancers, paving the way for rational treatment selection based on identified oncogenic drivers. DNA sequencing was first described in the 1970's by Sanger *et al*, who described a biochemical process that mirrors natural DNA synthesis[194]. Briefly, DNA fragments are amplified by polymerase chain reaction (PCR) and then sequenced through the addition of deoxynucleotides. Dideoxynucleotides, chain elongating inhibitors of DNA polymerase are added, generating DNA fragments of different sizes that can be separated by capillary electrophoresis, allowing the complementary base sequence to be interpreted. Through automating and developing this process further it was possible to complete the Human Genome Project in 2003 and introduce DNA sequencing as a clinical diagnostic tool. Next-generation sequencing (NGS) follows the same principles, but is a more fully automated process that allows millions of sequencing reactions to occur in parallel, hence its alternative name, massively parallel sequencing or high-throughput sequencing[195]. Sophisticated sequencing machines automate DNA amplification and then independently sequence each copy of the DNA fragment simultaneously, providing multiple reads of each fragment. This enables huge amounts of genomic data to be acquired in less time and cost. It is through this approach that it has been possible to sequence cancer genomes.

Cancer has been described as a disease of the genome, characterised by the presence of multiple mutational clones, driven by the accumulation of genomic changes that provide a selective advantage within the tumour environment[196]. The cancer genome will reflect the germline genomic profile of the individual in which the cancer has arisen and, somatic mutations, defined as acquired changes that are unique to the cancer cell. Paired examination of germline DNA is therefore recommended to correctly distinguish germline from somatic changes[197]. Processes through which somatic mutations may arise include exogenous or endogenous mutagen exposures or defective DNA repair[198].

Sequencing studies have revealed the considerable genetic diversity observed in tumours arising from the same primary sites in different individuals (intertumoural heterogeneity)

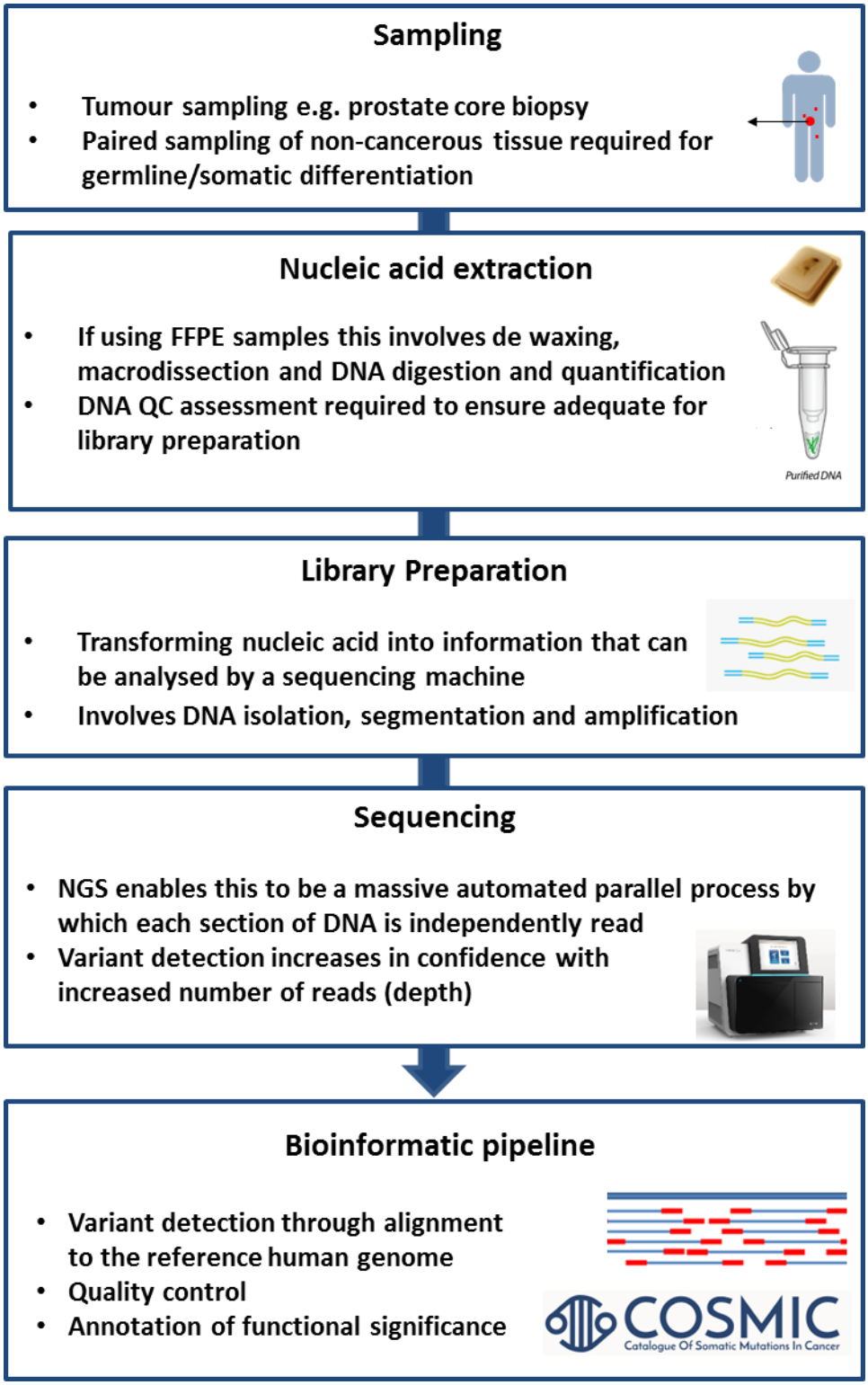
and within tumours (intratumoural heterogeneity)[199]. Somatic mutations may occur early in tumorigenesis, such that they are present in all tumour cells; referred to as clonal or truncal, subsequent somatic mutations are observed to develop and may follow a branched pattern of evolution, such that multiple sub-clones may co-exist, accelerating genetic variability[200,201]. Within this genomically diverse and dynamic environment, some mutations will be considered drivers of disease, capable of promoting tumour growth and others less so, referred to as passengers [202,203]. In PCa both spatial and temporal heterogeneity is observed, with different clonal populations present in different metastatic sites and changes observed over time in response to treatment-related pressures[35].

Added to this complexity, tumour samples e.g. prostate core biopsies, typically contain both cancerous and non-cancerous cells in varying proportions (referred to as tumour purity), this means that the DNA sequenced will contain germline variants present in the individual, together with a selection of somatic variants contained in the clones and sub-clones in the cancer cells captured by the biopsy. One of the advantages of NGS is that, providing each DNA segment is sequenced and read to a sufficient depth, it is sufficiently sensitive to detect sub-clonal mutations[195]. Complex computational methods that incorporate the number of reads, the copy number and proportion of tumour vs. normal cells, aim to infer clonal from sub-clonal mutations, although the functional and clinical significance of sub-clonal mutations is currently less clear[204].

Interpretation of this huge and complex data requires bioinformatic and genetic expertise. Data interpretation relies on a validated process of data processing that is referred to as the bioinformatic pipeline. These analysis algorithms function to impose filters to adhere to pre-defined quality standards and to ensure results are robust and reproducible[205]. Most bioinformatic pipelines will involve three key steps[206]; alignment of the sequencing data against the reference human genome in order to detect variants from 'normal'. Secondly, an assessment of the read quality to determine if the sequencing depth, that is the number of times a DNA segment has been read, is sufficient to be confident in distinguishing a sequencing error from a true variant. Thirdly, annotation of functional significance i.e. what is the likelihood that the variant detected is pathological? When processing cancer samples some pipelines will also include a comparison with the individual's germline DNA in order to confidently distinguish somatic from germline variants. The assessment of functional significance is informed by the type of mutation e.g. frameshift or stop-gain variants are more likely to result in a protein change, and therefore be significant. However,

reflecting that this is a rapidly developing field where knowledge is in constant flux, many pipelines will be linked with databases that collate published data describing cancer related somatic changes, such as the Catalogue of Somatic Mutations in Cancer (COSMIC), (http://cancer.sanger.ac.uk/cosmic) maintained by the Wellcome Sanger Institute[207].

Figure 26: Overview of Next-generation Sequencing



4.1.2 Genomic data obtained in prostate cancer cohorts

Increasingly complex and sophisticated integrated analyses have enabled comprehensive molecular profiling of tumour and patient tissue at the genomic, transcriptomic, proteomic and epigenetic level. Several, multi-academic collaborations such as that funded as part of the Stand Up To Cancer-Prostate Cancer Foundation (SU2C-PCF) translational programme, have been successfully undertaken[33,39]. These demonstrate that large-scale efforts to molecularly profile PCa cohorts are both feasible and informative; see **Table 45** for summary of characteristics of the cohorts profiled to date.

Sampling

Initially, sequencing studies utilised surgical prostatectomy samples, preferably fresh frozen as the optimal sample type to ensure sufficient amounts of high quality nucleic acid[30,31,34,38]. However, in order to profile patients with more extensive disease who are unsuitable for radical surgical treatment it was necessary to broaden this approach. Warm-autopsy studies permit multiple metastatic sites to be sampled, exploring the genetic changes observed in fatal mCRPC and illuminating the complex ways in which sub-clones compete and seed metastatic sites[31,35,36]. The development of patient-derived xenograph models from fatal mCRPC samples has further supported the investigation of recurrent genomic features including mutational burden and sub-clonal architecture[208]. As sequencing technology has improved, the required amount of extracted nucleic acid has decreased and this has enabled the study of patients with mCRPC, enrolled in protocols mandating fresh frozen metastatic biopsies[39,57]. However, whilst fresh frozen samples remain the gold standard for nucleic acid preservation, cost means that this is not routine clinical practice and recent efforts have focused on optimising nucleic acid extraction and NGS protocols for formalin-fixed material. The MSK-IMPACT cohort study provides the best example to date of where FFPE material has been used to sequence 451 patients using routinely available clinical samples, including prostate core biopsy material[40]. Optimising this approach will be important to determine if molecularly-directed therapies can be successfully implemented into existing routine PCa clinical pathways.

Sequencing approaches

NGS approaches have ranged from whole genome sequencing (WGS) to whole exome sequencing (WES), the latter are limited to the ~1% coding regions of the genome that include the majority of cancer related somatic changes[198]. These approaches can support discovery and unbiased profiling; alternatively, targeted assays (tNGS) that amplify and

sequence specific regions allow profiling of genes of prior interest e.g. those commonly mutated in cancer. In addition, through the collection of paired normal tissue e.g. whole blood or buccal saliva swabs, it is possible to sequence germline DNA to determine the prevalence of inherited aberrations in known cancer susceptibility genes such as Breast Cancer susceptibility gene 2 (*BRCA2*)[209,210].

Population characteristics

The majority of cohorts profiled to date have had either localised operable PCa or advanced heavily pre-treated or fatal mCRPC. There is relatively little evidence obtained from men with metastatic castrate-sensitive prostate cancer. It is hypothesised that the biology of *de novo* metastatic disease may be more similar to mCRPC than localised disease suitable for prostatectomy. This assumes that the somatic changes observed in mCRPC are not all in response to treatment-exerted selection pressures, but may occur as early events in tumorigenesis so may be present earlier, at first presentation of metastatic castrate-sensitive disease. These genetic features may correlate with aggressive disease, consistent with a *de novo* metastatic presentation and progression to CPRC. In the following sections I will summarise the published prevalence data focusing on the studies that have included men with metastatic disease, accepting that, with the exception of the MSK-IMPACT cohort, all sampled patients had advanced or fatal mCRPC.

Prevalence data

AR pathway aberrations are seen in 50-70% of mCRPC and include amplification or mutation of AR itself or associated genes such as *FOXA1*, an *AR*-associated transcription factor or *NCOR1/2* and a negative regulator of *AR*[33,35,39,40]. These somatic mutations are thought to occur as a result of ADT exerted treatment pressure and signal the molecular transition to the castrate-resistant state[179]. Activation of the Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-AKT pathway is seen in around a quarter of primary prostate cancers and up to half of mCRPC[38,39,211]. PI3K activation phosphorylates and activates AKT, itself negatively regulated by the tumour suppressor PTEN. AKT mediates the phosphorylation and activation of mTOR complex 1, a regulator of protein translation, synthesis, angiogenesis and cell cycle progression[212]. Dysregulation of this pathway occurs in multiple tumour types and can occur through several mechanisms; in PCa the most common mechanism is through PTEN loss or truncating mutations. However mutations, amplification and activating fusions involving PI3K subunits or AKT are also observed[33,39,40]. PTEN loss is associated with *ERG* rearrangement and in multi-regional sampling of

metastatic disease aberrations in *ERG* are consistent but PTEN loss demonstrates spatial discordance, suggesting PTEN loss is sub-clonal and may occur as a 'second hit' following *ERG* rearrangement[31,213,214].

The tumour suppressor gene *TP53* regulates the transcription of numerous downstream target genes involved in cell cycle arrest, DNA repair and apoptosis and thus when p53 activity is lost, cells lose the ability to control their growth and death[215]. After *AR* and the ETS family, mutations in *TP53* are the third most common somatic point mutation identified in metastatic prostate cohorts, occurring in 40-53%[30,36,39]. Analysis of matched archival and metastatic tissue has demonstrated *TP53* mutations to be concordant and present in all cancer cells, consistent with this being an early clonal driver mutation[40]. Cell cycle dysregulation may also occur as a result of aberrations within the cyclin/cyclin dependent kinase (*CDK*)/retinoblastoma (*RB*) axis, a critical modulator of cell cycle entry [202]. *RB1* loss is present in up to 20% of mCRPC and has been associated with neuroendocrine prostate cancer (NEPC), an aggressive subtype rarely identified in the hormone-naïve setting but present in up to 25% of lethal mCRPC [216]. Additionally, aberrations in cyclin-dependent kinases (CDKs) such as *CDK4*, *CDKN2A/B* and *CDKN1B* have been identified in 4-10% [39,40].

Table 45: Published cohorts: approach to sequencing and sampling

Reference	Cohort details	M0	nHNPC	mCRPC	Sequencing	Sampling
Barbieri *et al.*2012[34]	M0 CSPC suitable for prostatectomy	112		-	WES	Frozen tumour blocks sampled at RP and matched normal
TCGA 2015[30]	Predominant low-intermediate risk M0 CSPC	333		-	WES & WGS	Frozen tumour blocks sampled at RP and matched normal
Baca *et al.*2014[31]	Predominantly M0 CSPC suitable for RP; two M1 patients with NEPC	55		2	WGS	Frozen or FFPE tumour blocks sampled at RP and matched normal. 2 lymph node metastases (NEPE)
Taylor *et al.*2010[30]	Mixed cohort but predominantly M0 CSPC	181		37	Transcriptomic & CNA Exome sequencing (n=80)	181 sampled at RP with matched normal. Additional 37 metastatic samples
Beltran *et al.*2013[37]	Mixed cohort including: M0, mCSPC and mCRPC including 18 with NEPC	16	4	25	tNGS	FFPE samples: archival RP and prostate core biopsies
Grasso *et al.*2012[36]	Fatal mCRPC (rapid autopsy programme) and M0 HNPC suitable for prostatectomy	11		50	WES	Fatal mCRPC sampled at rapid autopsy RP from M0 subgroup
Cheng *et al.*2016[217]	Selected due to unusual clinical course, suspected clinical predisposition e.g. family history	29		13	tNGS	Fresh or archival FFPE samples (27 archival primary samples, 14 M1 tumour and 2 salvage RP samples
Kumar *et al.*2016[35]	Fatal mCRPC sampled at rapid autopsy			63	WES and gene expression	Multiple metastatic samples obtained at rapid autopsy
Robinson *et al.*2015[39]	Participants in mCRPC trials at academic centres	-		150	WES	Fresh frozen metastatic samples
Mateo *et al.*2015[57]	mCRPC eligible for olaparib trial (TOPARP-A) (also analysed as part of Robinson *et al*[39])	-		50	tNGS (DNA repair panel)	Fresh frozen metastatic tumour biopsies
Pritchard *et al.*2016[210]	Seven cohorts participating in clinical trials, autopsy or precision medicine programmes			692	tNGS (DNA repair panel)	Germline assessment using whole blood, buffy coat or buccal sampling.
Hussain *et al.*2017[218]	Sporadic mCRPC eligible for abiraterone +/- veliparib clinical trial	-		80	tNGS (DNA repair panel)	Fresh frozen metastatic biopsies
Castro *et al.*2017[219]	Sporadic mCRPC eligible for PROREPAIR-B	-		419	tNGS (DNA repair panel)	Germline only
Abida *et al.*2017[40]	Prospective profiling of pts attending MSK	103	135	211	tNGS	FFPE samples and blood for germline analysis

Key: CNA, copy number alteration; CSPC, Castrate-sensitive prostate cancer; FFPE, formalin-fixed paraffin embedded; RP, radical prostatectomy; M0, non-metastatic; mCSPC, metastatic castrate-sensitive prostate cancer; MSK, Memorial Sloan Kettering; NEPC, neuroendocrine prostate cancer; tNGS, targeted next-generation sequencing; TOPARP, Trial of Trial of PARP Inhibition in Prostate Cancer; WES whole exome sequencing, WGS, Whole Genome Sequencing.

The maintenance of DNA integrity is a crucial cellular function, which when impaired within cancer cells contributes to genomic instability, thus promoting the acquisition of further oncogenic mutations[220]. Of the numerous genes involved in DNA repair, *BRCA1* and *BRCA2* are the most widely studied and, together with Ataxia telangiectasia mutated gene (*ATM*), Partner and localizer of *BRCA2* (*PALB2*), *Rad51*, *Rad52*, Checkpoint kinase 2 (*CHEK2*) and Fanconi anaemia, complementation group A (*FANCA*) are components in the homologous recombination pathway[221-223]. The SU2C-PCF collaboration collated exome data from 1013 prostate cancers (including 680 primary and 333 metastatic tumours) and demonstrated 10% of primary and 27% of metastatic PCa harboured mutations in DNA repair genes[33]. Consistent with this relative prevalence, when comparing primary and mCRPC samples *BRCA2* was one of several genes to demonstrate significant enrichment[40]. This can be explained in one of two ways; somatic BRCA2 loss-of-function occurring as a result of treatment-related pressure as a molecular feature of CRPC, or alternatively as *BRCA2* mutations are associated with more aggressive disease, CRPC cohorts are enriched for this genetic subtype[224,225]. Evidence from matched archival and metastatic tissue would favour the latter explanation as somatic *BRCA2* mutations were all concordant, suggesting that this occurred early in tumorigenesis[40]. Comparative frequencies of non-*BRCA* HRD aberrations have not been reported. However, when the incidence of somatic mutations is compared between mCSPC and mCRPC, all are more prevalent in mCRPC (*ATM* 7% vs. 10%; *FANCA* 3% vs 7%, and *CDK12* 6% vs. 11%), suggesting these potentially occur later in tumorigenesis[40].

Microsatellite instability (MSI) is a phenotypic consequence of impaired DNA repair resulting in genetic hypermutation. MSI is associated with mutated mismatch repair genes (MMR) such as mutS homolog 2 (*MSH2*) and mutS homolog 6 (*MSH6*). MSI has been reported in 3-12% of mCRPC cohorts and preliminary data suggests an association with ductal adenocarcinoma, a rare non-PSA secreting subtype with distinct histopathological features and an aggressive clinical course[226]. This is suggested by an analysis of 9 cases of ductal carcinoma sampled at rapid autopsy where 4 had evidence of MSI[227]. Examination of paired normal tissue confirmed somatic mutations in DNA mismatch repair genes, although in contrast with colorectal and endometrial cancer, epigenetic silencing was not observed, instead complex structural rearrangements in *MSH2* and *MSH6* occurred with resultant protein loss confirmed by IHC. Multi-regional sampling confirmed MSI was present in all metastatic sites, and concordance was also shown in the two cases where paired archival and metastatic samples were available[228].

Other less frequent somatic alterations include those affecting the Wnt-B Catenin pathway, aberrant in 15-20% with mutations identified in adenomatous polyposis coli (*APC*), catenin beta-1 (*CTNNB1*) and ring finger protein 43 (*RNF43*)[39,40]. Mutations within the RAS-RAF-MAPK pathway are observed in 3-5% and include mutations in BRAF, KRAS, RAF1 and mitogen-activated protein kinase 1 (MAP2K1). Additionally, mutations in chromatin modifiers are seen in 2-12% with the highest frequencies observed in ETS-negative mCRPC; genes involved include mixed-lineage leukemia-3 (MLL3 also known as KMT2C) and CHD1[33,39,40].

In summary, the most common aberrant pathway mutations in metastatic prostate cohorts that are likely to be relevant to *de novo* metastatic disease are *TP53*, *PTEN-PI3K-AKT* and DNA repair. Of these, only DNA repair deficiency is currently thought to be therapeutically 'actionable' and therefore this remains the largest group for which there is a strong rationale to investigate a molecular-directed therapeutic strategy. We hypothesised that the prevalence of HRD in mCSPC would be less than observed in mCRPC, but more than observed in M0 disease.

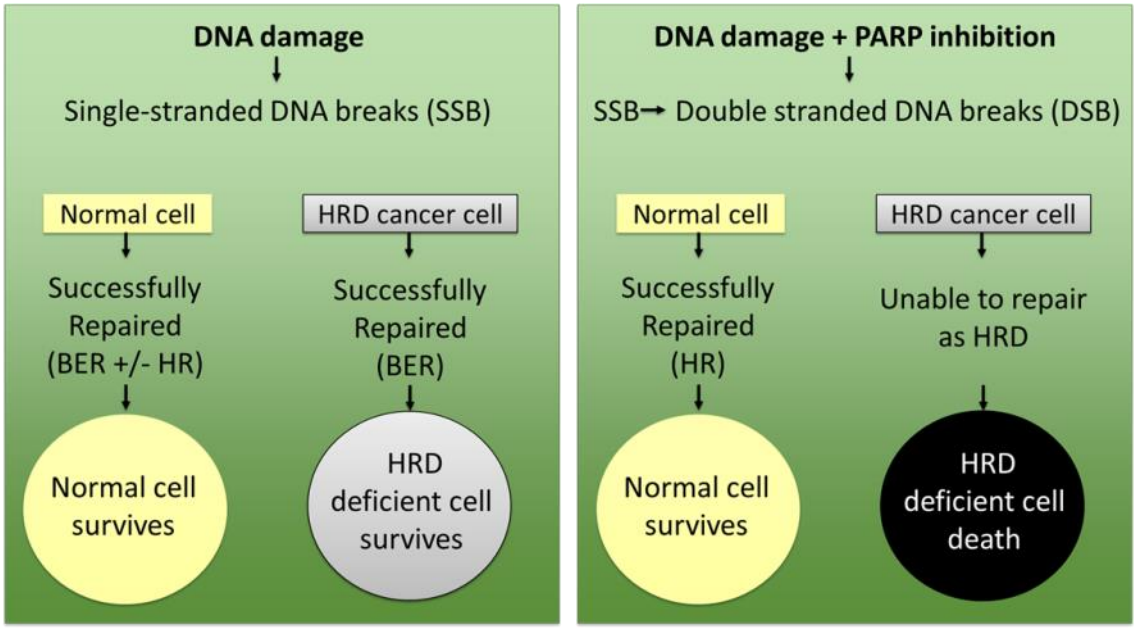
4.1.3 Rationale for therapeutically targeting DNA repair deficiency

DNA damage may lead to single strand breaks (SSB), double stranded breaks (DSB) or base modification[229]. SSB are common and may arise from exogenous factors such as reactive oxygen species; or endogenous factors such as base excision repair (BER) of damaged bases that may occur as a result of DNA topoisomerase 1 activity[230]. DNA repair may occur by several mechanisms; SSB are predominantly repaired by BER and to a lesser extent, nucleotide excision and DNA mismatch repair. BER is reliant on the PARP family of enzymes, which are essential for efficient DNA repair[231]. If left unrepaired, SSB become DSB during cellular replication. DSB are highly unstable and can induce mutagenesis or cell death therefore highly organised mechanisms of DNA repair are required; the most accurate and efficient of these is homologous recombination which competes with the error prone non-homologous end joining pathway (NHEJ)[230].

Homologous recombination repair is initiated via the recruitment of ATM which senses DSBs and promotes the formation of the MRN complex, containing MRE11, Rad50 and NBS1. ATM then plays a key role in phosphorylating substrates including BRCA1, or alternatively tumour-protein p53 binding protein 1 (TP53BP1) if NHEJ is being used. Which

pathway is selected depends in part on cyclin dependent kinases such as CDK12, which when active, favour BRCA1 activation[232]. Activated BRCA1 interacts with BRCA2 and PALB2, and together they promote Rad51 and replication protein A to initiate DNA repair. DNA is repaired using the homologous region of the sister chromatin as the replicative template, helping to avoid the errors of competing NHEJ pathway[229]. Homologous recombination is a complex, highly accurate pathway that involves many genes. The exact contribution of each gene is incompletely understood and there is likely to be some degree of redundancy. Loss of function of any of the genes critical to homologous recombination is predicted to result in HRD leading to a reliance on alternative, less efficient mechanisms of DNA repair such as BER, which are all PARP dependent, thus providing the rationale for therapeutic PARP inhibition in HRD cancers[233,234].

Figure 27: Synthetic lethality



Synthetic lethality: exploiting deficiencies in DNA repair: Single-stranded DNA breaks (SSB) can be repaired using base end repair (BER) and homologous recombination. Repair of double stranded DNA breaks (DSB) requires homologous recombination. Homologous recombination deficient (HRD) cancer cells are sensitive to PARP inhibition which leads to accumulating DNA damage and selective cancer cell death

Table 46: Selected genes involved in Homologous recombination

Gene	Name	Function
ATM	Ataxia Telangiectasia-mutated	Encodes ATM , a protein kinase that senses DNA damage and phosphorylates key substrates involved in DNA repair including BRCA1, checkpoint kinase CHK2 and other checkpoint proteins [235,236]
BARD1	*BRCA1* associated RING domain 1	The BARD1 protein binds to and interacts with BRCA1[237]
BRCA1	BReast CAncer susceptibility gene -1	Encodes, BRCA1 a nuclear phosphoprotein that assists in 5' to 3' resection of DSBs and loading of Rad51[238]
BRCA2	BReast CAncer susceptibility gene -2	Encodes BRCA2 which regulates the activity of Rad51 by binding to the site of DNA damage and encasing it in a protein sheath, the first step in the DNA repair process[238]
BRIP1	BRCA1 interacting protein C-terminal helicase 1	The BRIP1 protein interacts and forms a complex with BRCA1[239]
CDK12	Cyclin dependent kinase 1	Regulates expression of *BRCA1* and other genes involved in DNA repair[240]
CHEK2	Checkpoint kinase 2	A checkpoint protein which halts cell cycle progression in response to DNA damage and also interacts and phosphorylates BRCA1[241]
NBN	Nibrin	Nibrin (protein product of *NBN* gene) forms a complex with MRE11A and Rad50 (MRN complex) that interacts with the *ATM* gene[242]
PALB2	Partner and localizer of BRCA2	PALB2 recruits BRCA2 and Rad51 to the site of DNA breaks[243]
Rad51	Rad51 recombinase	Interacts with BRCA2 and BRCA1; in turn activation is controlled by BRCA2, loss of this control is thought to be key event in genomic instability and tumourigenesis[244]
Rad51B	Rad51 paralog B	Member of the Rad51 protein family, Rad51B forms a stable heterodimer with Rad51C and helps sense DNA damage[245]
Rad51C	Rad51 paralog C	Forms a sub-complex Rad51B:Rad51C (part of BCDX2) which facilitates phosphorylation of the checkpoint kinase CHEK2 leading to cell cycle arrest and homologous recombination activation[246]
Rad51D	Rad51 paralog D	Part of the Rad51 paralog protein complex BCDX2, assists with Rad51 stabilization [247]
Rad54L	Rad54L	Binds to double-stranded DNA and facilitates homologous recombination repair[248]

Key: BCDX2, Rad51B-Rad51C-Rad51D-XRCC2 Complex

4.1.4 Evidence of predictive effect

Of the many genes involved in homologous recombination, *BRCA1* and *BRCA2* have been the most studied. Data obtained in ovarian, breast and prostate cancer demonstrate that *BRCA1* and *BRCA2* mutations have the strongest evidence of predictive effect to PARP inhibition[57,249-252]. Clinical evidence that genetic faults in non-*BRCA* HRD genes have the same predictive effect has not been directly sought in ovarian and breast cancer. However, the therapeutic relevance of HRD arising from mutations in non-*BRCA* HRD genes has been shown in the study of platinum responses in ovarian cancer[253]. In a study of 390 patients with advanced ovarian cancer, germline or somatic mutations in one or more of 13 HRD genes (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK1, CHEK2, ABRA1, MRE11A, NBN, PALB2, Rad51C* and *Rad51D*) was associated with platinum sensitivity (p=0.0002). As platinum is a DNA-damaging chemotherapy HRD tumours are predicted to exhibit similar therapeutic sensitivity. This therefore may be considered indirect evidence that HRD arising due to mutations in genes other than *BRCA1* or *BRCA2* are therapeutically relevant.

ATM loss is predicted to result in PARPi sensitivity reflecting its important role in homologous recombination repair, see **Table 46**. As *ATM* mutations are relatively rare in ovarian cancer there is limited clinical evidence in this setting, however recent *in vitro* data in *ATM*-mutant colorectal cancer demonstrates PARP sensitivity[254]. Pre-clinical data has also shown *NBN*, *Rad51*, *CHK2* and *PALB2* mutations all to predict PARP sensitivity *in vitro*[255-260]. Preliminary clinical evidence for activity in the broader HRD group in PCa is also provided by the TOPARP trial, a phase 2 study of olaparib in mCRPC. Responses were observed in 4/6 patients with an *ATM* mutation, 2/3 with *FANCA* mutation, 1/2 with *CHEK2* mutation, 2/2 *PALB2* mutation, 1/1 *NBN* mutation; although it should be noted that both patients who had a *FANCA* and *CHEK2* mutation also had a *BRCA1* or *BRCA2* mutation[57].

In summary, mechanistically the presence of HRD occurring through germline or somatic mutation in a relevant gene predicts synthetic lethality with PARP inhibition. Homologous recombination is a complex, multi-gene pathway and the functional significance of individual mutations within known genes remains incomplete. It is also possible that known genes involved in homologous recombination interact with other genes and pathways, meaning that additional genetic aberrations may impact on homologous recombination, or redundancy may rescue this function in the presence of mutations predicted to be significant. The strongest evidence of a predictive effect is currently seen for *BRCA1* and

BRCA2 as these have been predominantly used as biomarkers in the clinical evaluation of PARPi to date, summarised in the sections below.

4.1.5 Initial evaluation of PARP inhibitors in ovarian and breast cancer

Ovarian data

The clinical activity of PARP inhibition was first shown in BRCA-associated ovarian and breast cancer. Olaparib, rucaparib and niraparib have all received regulatory approval for use in platinum-sensitive ovarian cancer. Olaparib was the first to be licenced in BRCA-mutated (BRCA-m) platinum-sensitive ovarian cancer based on the results of Study 19, which showed a significant improvement in PFS, 8.4 months vs. 4.8 months (HR 0.35 95% CI 0.24-0.49)[261]. A subsequent retrospective analysis demonstrated the largest benefit in BRCA-m arising as a germline or somatic loss[262]. BRCA status was assessable in 254/265 patients and the mutational prevalence was 51%. The treatment effect in the BRCA-m subgroup was HR 0.12 (95% CI 0.10-0.31), compared with BRCA-wild-type (BRCA-WT); HR 0.54; (95% CI 0.34-0.85).

It is hypothesised that observed benefit in BRCA-WT cases may be explained by functional HRD occurring due to epigenetic silencing of *BRCA1* or *BRCA2* or inactivation of other genes involved in homologous recombination[263]. In support of this, distinct mutational signatures associated with *BRCA1* and *BRCA2* mutations are also observed in BRCA-WT samples and developed as biomarkers of "BRCA-ness" or HRD[198]. These algorithms assess genomic features of instability as a marker of HRD, including loss-of-heterozygosity (LOH)[264], telomeric allelic imbalance[265] and large-scale state transitions[266]. Foundation Medicine provides an assessment of LOH as part of Foundation One, whilst Myriad Genetics have incorporated all three into their commercialised assay myChoice®. Both niraparib and rucaparib have been evaluated in trials designed to evaluate these biomarkers of HRD, building upon the broader benefit suggested in Study-19, although as HRD is defined differently in all trials, these data are not directly comparable. An ongoing non-randomised phase II trial will also evaluate olaparib in four cohorts aiming to explore this further; germline BRCA (gBRCA) mutant, somatic BRCAm and gBRCA-WT, HRD positive as defined by myChoice and a further HRD negative cohort (NCT02983799).

Rucaparib is approved as a treatment and maintenance therapy for BRCA-m (germline or somatic) platinum-sensitive ovarian cancer. Evidence of activity in the relapsed setting was

shown by the results of the ARIEL2 study. This open-label phase 2 trial enrolled 206 patients and assessed PFS within three cohorts evaluating different biomarker enrichment strategies: BRCA mutant (n=40), BRCA-WT and high LOH (n=83), and BRCA WT and LOH low (n=71); it was not possible to determine LOH status in 12 cases. PFS was 12.8 months (95% CI 9.0-14.7) in the BRCA-m subgroup, 5.7 months (95% CI 5.3-7.6) in the high-LOH group and 5.2 months (95% CI 3.6-5.5) in the LOH low subgroup. ARIEL 4 is an ongoing post-registrational confirmatory study in this setting. ARIEL-3 demonstrated that rucaparib is also beneficial in the maintenance setting. This placebo controlled phase 3 RCT enrolled 564 patients analysed in three cohorts using a stepdown approach: BRCA-m (n=196); high-LOH, which may include BRCA mutant, (n=354), and the entire ITT i.e. molecularly unselected. Consistent with previous results, the largest benefit was observed in the BRCA-m group, HR 0.23 (95% CI 0.16-0.34) p<0.0001. A significant improvement in PFS was also shown in the high LOH subgroup; HR 0.32 (95% CI 0.24-0.42) p<0.0001. When the BRCA-m group are excluded (as they overlap with high-LOH and may be driving this effect) we continue to observe benefit in the BRCA-WT group, consistent with the results of Study-19. However when the treatment effect is examined in the BRCA-WT group the predictive ability of LOH appears less clear; BRCA-WT LOH-high (HR 0.55; 95% CI 0.35-0.89) p=0.0135 and BRCA-WT-LOH-low (HR 0.47; 95% CI 0.31-0.71) p=0.0003. Therefore the utility of LOH remains uncertain, importantly threshold values continue to require refinement, and are yet to be validated outside of ovarian cancer[267].

Niraparib is the only PARPi to receive approval for non-BRCA mutated, platinum-sensitive ovarian cancer, based on the results of the NOVA trial[268]. In total 553 patients were enrolled and split into two efficacy populations based on the results of gBRCA testing; gBRCA mutant (n=203) and non-gBRCA mutant (n=350). Significant PFS benefit was shown in both gBRCA mutant, HR 0.27 (95% CI 0.17-0.41) p<0.01 and non-gBRCA-mutant cancers; HR 0.38 (95% CI 0.24-0.59) p<0.01, supporting the regulatory approval in an unselected population. Further exploratory subgroup analyses were performed and confirm that germline and somatic *BRCA1* or *BRCA2* mutations appear to confer the same predictive effect, gBRCA-mutant HR 0.27 (95% CI 0.17-0.41) and somatic BRCA-mutant 0.27 (95% CI 0.08-0.90). Prior to the primary analysis a protocol amendment required submission of tumour samples for HRD analysis, assessed using myChoice®, classifying non gBRCA-mutant cases as HRD positive and negative and again, benefit was observed in HRD negative cases: HR 0.58 (95% CI 0.36-0.92) p<0.01.

Breast Cancer data

PARPi have also been shown to be active in BRCA-m metastatic breast cancer, with two large positive phase III trials reported in 2017. The OlympiAD trial randomised 302 patients with gBRCA-mutant, HER-ve metastatic breast cancer to receive olaparib or physicians choice therapy[252]. Blinded central review of rPFS was required given the open-label design and demonstrated a significant improvement; 7.0 vs. 4.2 months (HR 0.58; 0.43-0.80) p<0.001. This led to regulatory approval of both olaparib in this indication, and a companion diagnostic, Myriad Genetic BRCA Analysis Companion diagnostic. The recently reported EMBRACA trial has also shown a comparable PFS gain with talazoparib in BRCA-m metastatic breast cancer; 5.6 months vs. 8.6 months (HR 0.54; 0.41-0.71, p<0.0001)[269].

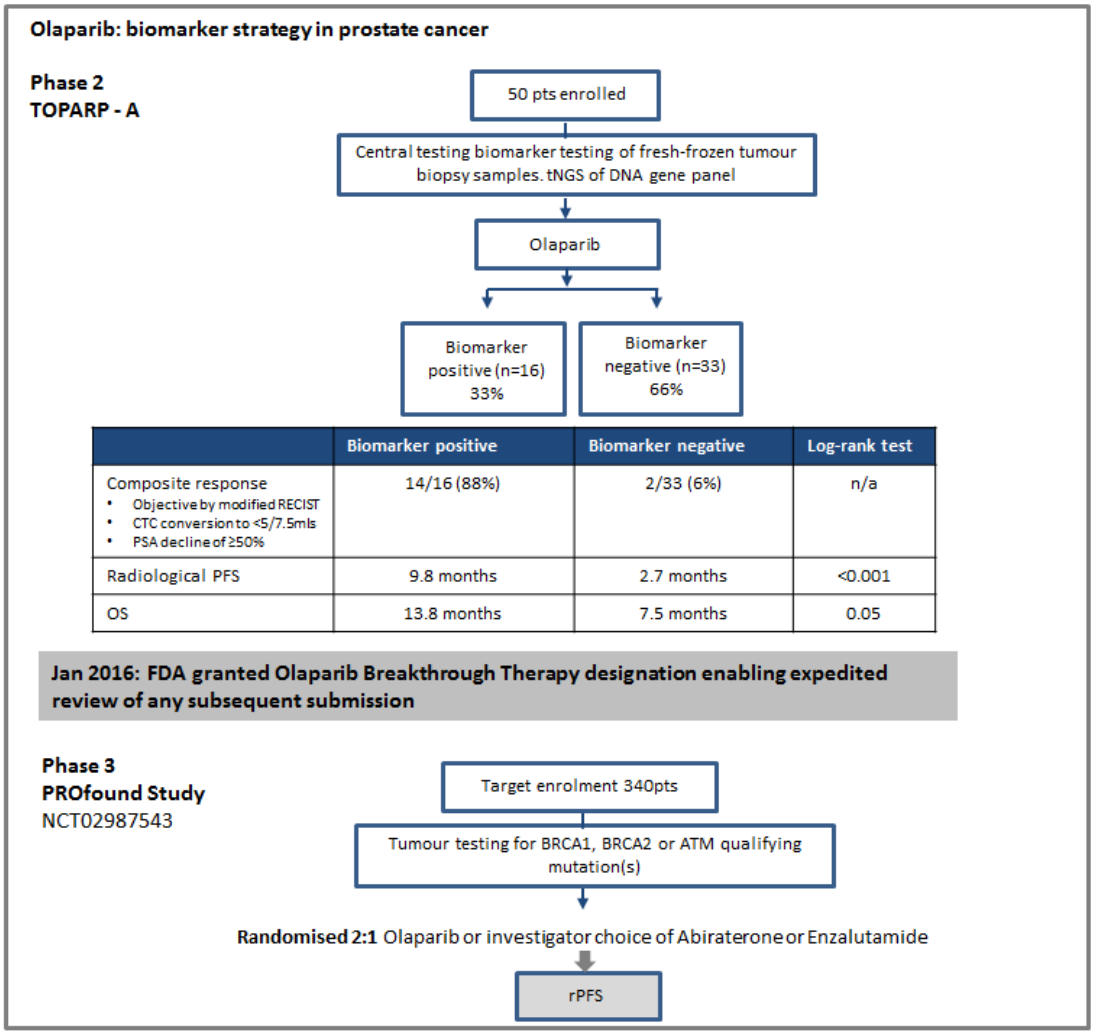
4.1.6 Rationale for PARP inhibitors in prostate cancer

A proportion of prostate cancers are associated with gBRCAm; for men ≤65 years BRCA2 mutations confer a 8.6 fold increased risk and *BRCA1* a 3.4 fold increased lifetime risk of developing prostate cancer[270,271]. The demonstration that BRCA2 deficient cells are selectively sensitive to PARPi provided the rationale to include PCa in the range of BRCA-associated cancers eligible for the early phase PARPi trials[272,273]. AR activity has been shown to be linked with DNA repair. PARP1 is recruited to sites of AR activity where it regulates transcriptional function[274]. AR signalling has been shown to promote homologous recombination through facilitating MRN foci formation and ATM activation[275]. Therefore ADT can be expected to inhibit homologous recombination, requiring a shift to other less efficient and PARP-dependent mechanisms of DNA repair. It is proposed that this may explain the observed synergy between ADT and RT, another DNA damaging therapy which induces DSBs[276]. The same mechanism is thought to explain the *in vitro* and *in vivo* synergy of bicalutamide or enzalutamide with olaparib, and the demonstration that “lead in” with enzalutamide followed by PARPi suppressed HRD gene expression and promoted DNA damage-induced cancer cell death *in vivo* prostate cancer models[275,277]. Together, these data provide a strong pre-clinical rationale for the evaluation of PARPi in addition to ADT in PCa.

Early phase trials that sought to evaluate PARPi in gBRCA-m cohorts provided the first evidence of activity in BRCA-m prostate cancer. In a phase I trial eight patients with BRCA-m mCRPC were treated with olaparib and RECIST responses were observed in half[250]. In a second phase I trial, 3/60 patients enrolled had BRCA-m mCRPC, and biochemical, clinical or radiological responses were all seen. A durable treatment response was observed in one

BRCA2 mutant case, who remained on treatment for over a year having experienced a PSA response of >50% associated with resolution of bone metastases[249]. Supported by these data, several phase 2 trials were initiated in prostate cancer, as summarised in **Figure 28, Figure 29 and Figure 30.**

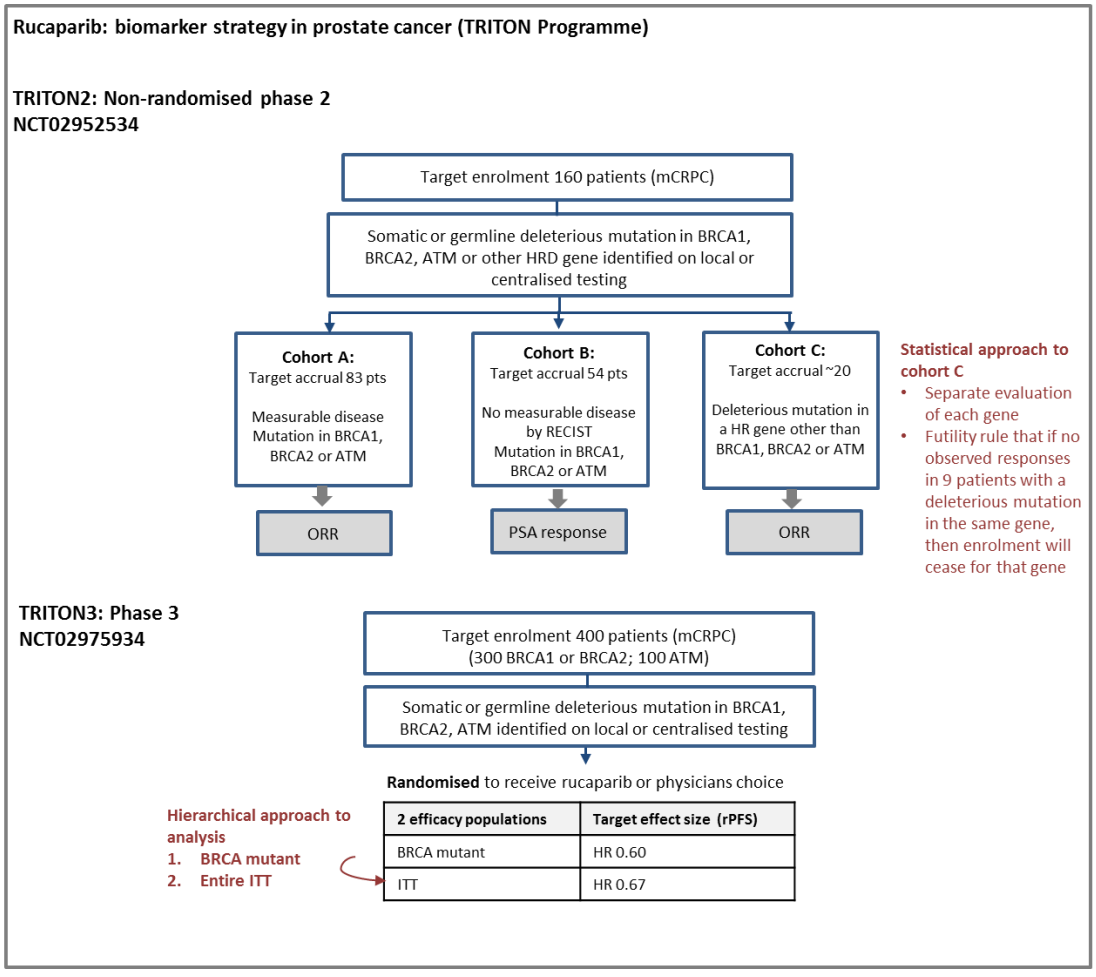
Figure 28: Olaparib: summary of ongoing evaluation in prostate cancer



When seeking to identify the subpopulation of PCa hypothesised to benefit most from PARPi, several differences exist in comparison to other BRCA-associated cancers that have led to a different biomarker enrichment approach. Firstly, platinum sensitivity may be considered a clinical biomarker likely to correlate with PARPi sensitivity, as a subsequent DNA-damaging therapy seeking to exploit the same biological vulnerability. Platinum sensitivity has been incorporated into all selection strategies for PARPi trials in ovarian cancer, and used as a surrogate for HRD sensitivity when developing biomarkers of HRD[265]. However, as platinum is not a standard treatment for PCa it cannot be used in the same way. Secondly, the observed prevalence of *BRCA1* and BRCA2 mutations in ovarian cancer is

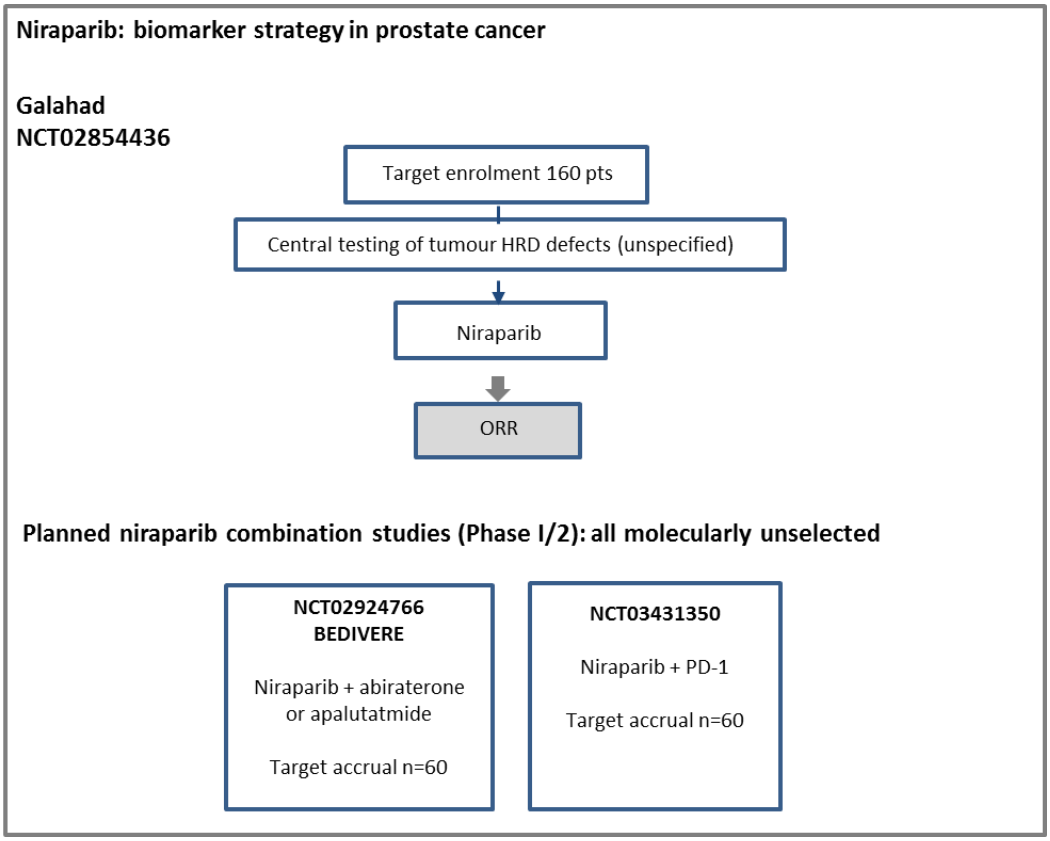
33-51%, much higher than in PCa. These differences help explain the adaptive 2-stage approach to biomarker selection adopted in TOPARP, a phase II evaluation of olaparib in mCRPC. Initially, an unselected population were recruited (n=50) to part A, all of whom underwent biomarker assessment using mandated fresh frozen biopsies obtained prior to trial entry. Biomarker assessment was not limited to *BRCA1* or *BRCA2*; instead a tNGS panel including 12 genes involved in homologous recombination was assessed. 33% of those enrolled were defined as biomarker positive based on a deleterious mutation in one or more HRD genes. High specificity was demonstrated for this approach. 14/16 biomarker positive patients responded, compared with 2 out of 33 biomarker negative patient. As detailed in Section 4.1.4, the strongest evidence of predictive effect was in *BRCA2*, *BRCA1* and *ATM*, in total 13/16 patient responded. Lack of response was observed where monoallelic deletions were present supporting the observations of others that biallelic loss is required to be functionally relevant[57,278]. This biomarker strategy has informed the design of the TOPARP-B trial which aims to recruit 88 participants with HRD-mCRPC (NCT01682772) and has influenced several other ongoing phase II trials.

Figure 29: Rucaparib: summary of ongoing evaluation in prostate cancer



The TRITON programme aims to evaluate rucaparib in HRD deficient mCRPC. TRITON 2 is enrolling three cohorts in order to evaluate activity in the *BRCA1*, *BRCA2*, *ATM* subset, as the group with the highest prior evidence of predictive effect. Additionally, preliminary evidence for activity in a broader group defined by predicted pathogenic mutations in one of 15 HRD genes listed in **Table 46** is also being sought in cohort C, see **Figure 29**. A phase II trial evaluating niraparib in a broader HRD-group of mCRPC, defined using a company specific assay has been initiated and results are awaited (NCT02854436).

Figure 30: Niraparib: summary of evaluation in PCa



In addition, PARPi are being evaluated in combination with checkpoint inhibitors or second-generation AR-targeted agents. The combination of PARPi and immune checkpoint inhibition is supported by the observation that HRD tumours are associated with increased tumour infiltrating lymphocytes (TILs) and may exhibit an immunosuppressive phenotype, characterised by upregulation of Programmed death-ligand 1 (PD-L1) expression[279]. This has been observed when comparing immune cell marker expression in ovarian cancer, which is higher in BRCA-m compared with BRCA-WT tumours[280]. Similarly, *BRCA1* associated triple negative breast cancers have increased TILs and overall mutational burden compared with *BRCA1*-WT tumours[279]. The suggestion that this subset may benefit from checkpoint inhibition is further supported by the demonstration that *BRCA2* mutations correlate with

responses to anti-PD-1 agents in melanoma[281]. The recent demonstration of synergy between AR signalling and HRD provides a rationale to investigate the combination with second-generation AR-targeted agents[275,277]. Should this combination be shown to be safe, this strategy could also be evaluated in the castrate-sensitive setting as abiraterone has recently been shown to be an alternative SOC to docetaxel. See **Appendix G AB:** Table 78 - **AB:** Table 80 for full list of PARPi trials in PCa.

In summary, whilst the clinical data in metastatic breast cancer is limited to gBRCA-mutant cases, there is clear evidence of a broader effect in ovarian cancer. We consistently observe the greatest benefit in BRCA-m cancers, and germline and somatic mutations appear functionally similar and to confer a similar predictive effect[268,282]. Within ovarian cancer, non-BRCA mutated cancers may also be effectively treated by PARPi but the clinical utility of biomarkers of HRD is yet to be convincingly shown, and as such regulatory approval within this biomarker-defined group has not been achieved. In comparison with other BRCA-associated cancers, hereditary PCa is rare meaning biomarker strategies that are limited to germline mutations are expected to fail to identify the entire population hypothesised to benefit. This means tumour-based assessments that capture both somatic and germline mutations are preferable. The evidence from TOPARP supports a tNGS approach that includes several of the genes known to be involved in homologous recombination. Finally, the biomarker strategies adopted in the pivotal trials have relied upon collaboration between pharmaceutical companies and commercial sequencing providers and several companion diagnostics have been approved as a result. Whilst regulatory assessment of predictive biomarkers may be expected to ensure high-quality standards are met, this pattern of joint regulatory submission of drug and companion diagnostic adds complexity to trial design and data use. From the perspective of an academic-led trial, such partnerships necessitate collaborations with multiple industry partners and can mean the sequencing data is commercially sensitive. Lastly, when considering how best to evaluate PARPi in prostate cancer, the commercialisation of biomarkers of HRD will likely mean that this population is defined differently across different trials which may risk delaying regulatory approval in this expanded indication, if shown to be effective.

4.1.7 Evaluating PARP inhibitors: trial design considerations

When considering how best to design and implement a randomised evaluation of a PARPi in mCSPC, biomarker prevalence was identified as the key factor in determining feasibility and the optimal approach. Prevalence impacts on the number needed to screen, accrual rate, recruitment duration and required number of participating screening sites. When addressing this question within a MAMS platform, the prevalence also determines the likely impact on the accrual and generalisability of other co-recruiting comparisons. When modelling the scenarios within STAMPEDE several trial designs were modelled and based on current recruitment rates, the minimum feasible prevalence was estimated at 10%, as shown in **Figure 31** and **Figure 32**.

Part of the rationale to investigate molecularly-selected PCa treatments in the first-line setting is the proximity to the time of diagnostic sampling. Although, to avoid repeat sampling, as required in many other CRPC trial protocols, it is necessary to demonstrate that the residual diagnostic FFPE tumour tissue yields sufficient DNA required for tNGS. Test failure rates inflate screening numbers and are therefore an important component in the feasibility assessment. Obtaining data in representative clinical samples was identified as a second aim that could be addressed through analyses of retrospectively collected samples from existing STAMPEDE participants with metastatic disease at trial entry.

Biomarker–selected trials that limit recruitment to one molecularly-defined cohort risk high screen failure rates which may negatively impact on participant and investigator enthusiasm, whilst incurring high screening costs. This was part of the rationale to embed this question within the STAMPEDE study, aiming to have an inclusive trial platform in which all participants may be eligible for randomisation, regardless of biomarker status. To be eligible to join the transdermal comparison or the metformin comparison participants must be randomised within 8 or 14 weeks of starting anti-androgens respectively. This therefore means the turnaround time for biomarker analysis becomes a critical rate limiting step and an important consideration in defining the duration of ADT permitted before biomarker screening. In turn, the eligibility criteria need to be workable within the current clinical pathway if the required screening accrual is to be achieved. Currently the vast majority of patients commence ADT before being approached about STAMPEDE trial participation and most have received around 6-9 weeks ADT exposure prior to randomisation; this means biomarker-screening needs to be completed within 4 weeks.

Piloting this process and testing out the necessary infrastructure required to implement this was recognised as an important part of the feasibility assessment.

Another design consideration relates to the prognostic impact of the biomarker as this determines whether it is valid to use the single control group within a MAMS platform. Data obtained from two large PCa cohort studies has shown gBRCA-m are associated with a more aggressive clinical course suggesting that a separate control arm may be needed to distinguish prognostic from predictive effect. In an analysis of 2019 patients with PCa enrolled in either the United Kingdom Genetic Prostate Cancer Study (UKGPCS) or Epidemiological Study of *BRCA1*/2 mutations Carriers (EMBRASE), g*BRCA1*-m or g*BRCA2*-m were shown to be independently prognostic of cancer-specific survival (HR 1.8; 95% CI 1.1-3.5; p=0.015)[224]. Recent analyses have suggested that germline defects in *ATM* may confer a similar negative prognostic effect. In a retrospective case-case study of 313 patients with lethal PCa and 486 patients with low-risk localised prostate cancer, germline DNA analysis demonstrated a significantly higher incidence of *BRCA1*, *BRCA2* or *ATM* mutations in those who died of their disease. Survival analyses in the entire cohort adjusted for race, age, PSA and Gleason score at presentation demonstrated the presence of a germline aberration in one or more of these three genes was an independent predictor of lethal PCa (HR 2.13; 95% CI 1.24-3.66; p0.004) [283]. Further support for this is provided by the retrospective review conducted as part of the PRO-REPAIR cohort study, which showed g*BRCA2*-m were associated with earlier progression to CRPC and shorter prostate cancer-specific survival[209].

Evidence for the prognostic significance of aberrations in other HRD genes is more limited. A published international collaboration reported outcomes in 390 patients with metastatic prostate cancer who had undergone germline analysis as part of various clinical trials or precision medicine initiatives. 60 were found to have germline aberrations in *BRCA2*, *BRCA1*, *ATM*, *CHEK2*, *PALB2* or *Rad51D*. The median survival from time of castrate-resistance was comparable in the gHRD-mutated group 3.0 years (IQR 2.4-5.6) vs. the gHRD-non mutated group 3.2 (1.7-5.5 years). Although likely underpowered, in a multivariable analysis adjusted for other known prognostic factors, the presence of germline HRD mutation was not shown to be statistically significant: adjusted HR 0.93; 95% CI 0.63-1.37; p=0.72)[284]. Within the PRO-REPAIR study 9.1% were found to have germline HRD, however the prognostic effect appeared driven by the *BRCA2*-m subset (3.3%)[209]. On the basis of these data that suggest germline aberrations in *BRCA1*/2 and *ATM* are negatively prognostic, and in the absence of data to elucidate the prognostic impact of

somatic aberrations, the proposal is that PARPi randomisations should be compared against a separate biomarker positive control arm to enable predictive and prognostic impact to be distinguished.

There are two clear advantages of evaluating biomarker-selected comparisons within an adaptive trial platform. Firstly, biomarker specificity can be tested through opening a randomised comparison within an unselected group. Where initial evidence for predictive effect is judged sufficient to support an enrichment strategy, this assessment may be undertaken once activity has been demonstrated at a pre-planned analysis of futility. Early stopping rules may be useful in the unselected population i.e. through defining different futility parameters. The need to undertake this assessment should be driven by data external to the trial, for example evidence that PARPi may benefit a broader group.

Secondly, as exemplified by other biomarker-stratified trial platforms such as FOCUS-4 and the National Lung Matrix Trial (Matrix), efficiencies can be gained through utilising the trials' infrastructure to address multiple biomarker-selected questions. These can be addressed through one centralised cost-efficient biomarker-assessment and multiple pharmaceutical collaborations can help reduce the cost of screening. However the feasibility and design implications of this strategy depend on the overlap between biomarker-defined subgroups. As has been shown to be the case in colorectal and lung cancer, sub-clonal putative aberrations may co-exist leading to uncertainty as to which therapy should be selected and requiring hierarchical systems to be established. Furthermore, HRD cancer may be hypothesised to acquire and tolerate other genetic aberrations at a higher frequency, increasingly the likelihood of overlap. Comprehensive genomic profiling within this disease setting is required to known if it is feasible to address multiple biomarker-selected comparisons within one protocol.

In summary, analysis of clinically representative samples from men with mCSPC enrolled in STAMPEDE can provide valuable genomic profiling data, assess the feasibility of addressing molecular biomarker-treatment pairings in this disease setting and inform how these could potentially be evaluated within a MAMS trial platform in the future.

Figure 31: Provisional trial design – biomarker prevalence 14%

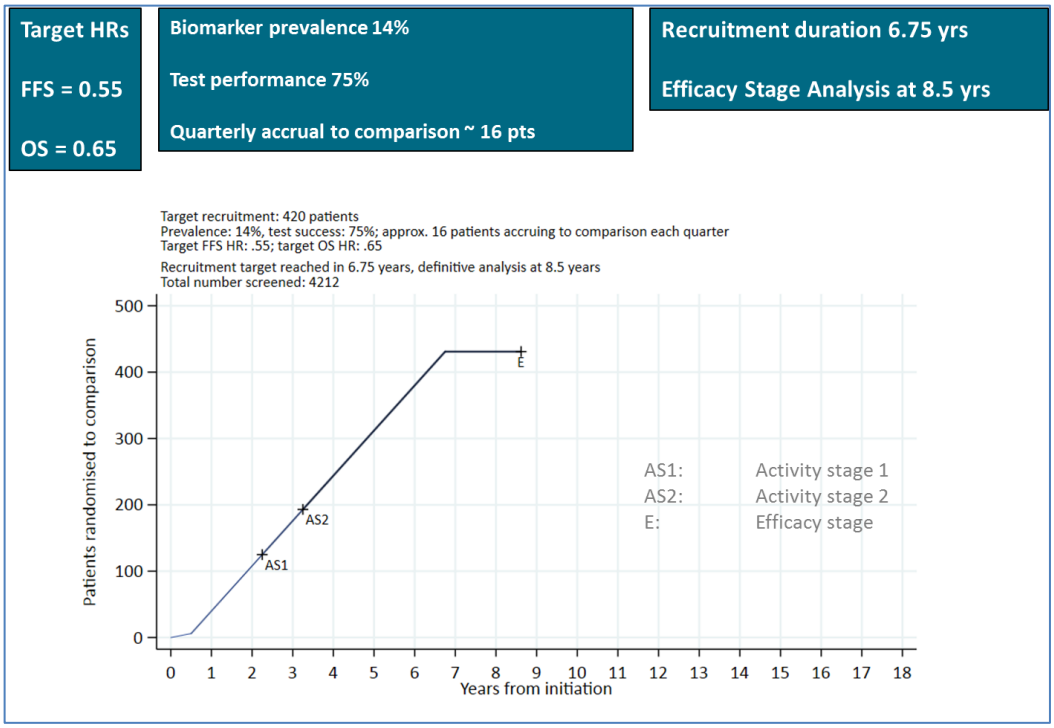
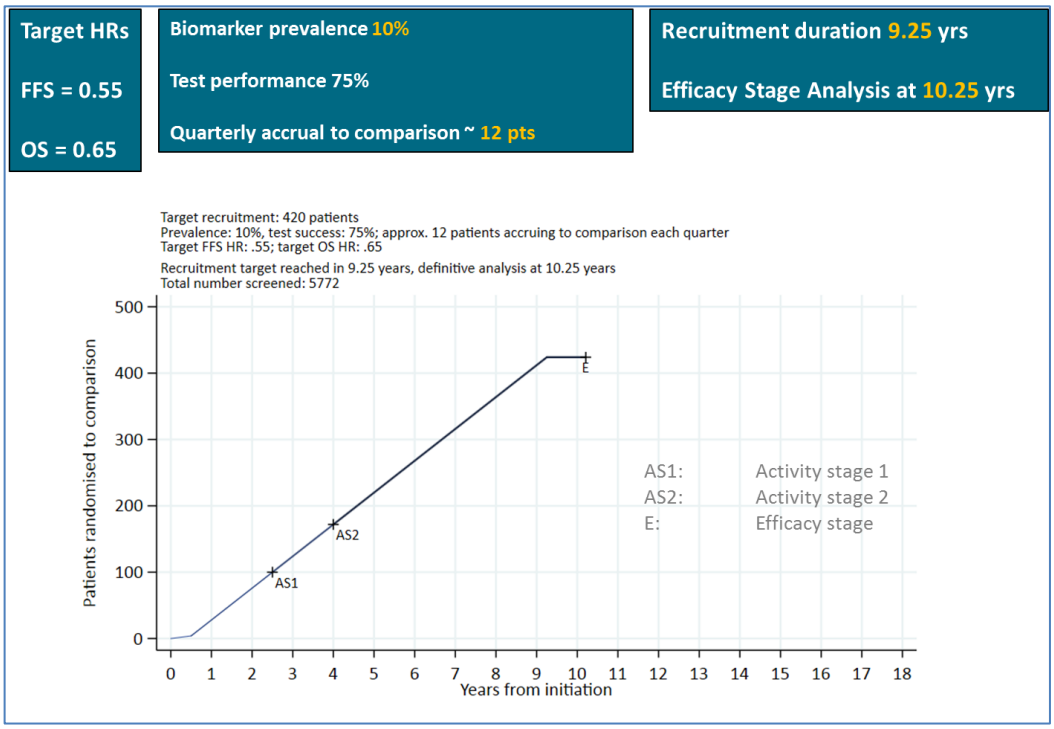


Figure 32: Provisional trial design – biomarker prevalence 10%



Provisional trial designs: The minimum biomarker prevalence required to achieve a efficacy analysis within around 10 years is 10% (allowing for a test failure rate of up to 25%). These projected scenarios are based on 100 patients joining STAMPEDE per month, 65% of patient having metastatic disease at trial entry and 80% of these accessing screening. Therefore 52 patients per month are eligible for screening. Given these parameters the total number needed to screen is 5772 and the recruitment duration is 9 years. If the prevalence if 14%, the accrual period is reduced to 6.75 years and the efficacy analysis is projected within 8.5 years. The total estimated required sample size is around 420 patients, targeting a survival difference of HR 0.65.

4.1.8 Research aims

In this chapter I will describe a feasibility and prevalence study undertaken to address the following three research questions:

1. Is it feasible to molecularly profile mCSPC through analysing remaining FFPE diagnostic tissue using tNGS assays?
2. What is the prevalence of HRD in mCSPC?
3. What is the prevalence of other putative actionable mutations and how do these groups overlap?

4.2 Methods

This study was undertaken through retrieving remaining diagnostic PCa samples stored as FFPE tumour blocks from a selection of NHS sites, in collaboration with the Wales Cancer Bank (WCB), the trial designated biobank. Funding was provided by Clovis Oncology who supported the costs of sequencing and provided a grant to the MRC CTU at UCL to support sample retrieval. Two analyses were performed in collaboration with two different commercial sequencing providers. Foundation Medicine (FM) sequenced all samples in analysis one and Almac Diagnostics (Almac) sequenced all samples in analysis two.

All STAMPEDE participants recruited since 2006 have been asked to consent to the use of remaining diagnostic PCa tissue in additional analyses. These projects require separate ethical approval and review by the trial oversight groups. I wrote a translational protocol that described these analyses and obtained ethical approval from the West Midlands REC (16/WM/0188), see **Appendix F Biomarker Development Protocol** and **Appendix G Confirmation of ethical approval**. This project was also approved by the STAMPEDE Biological Research Group, a subgroup of the TMG.

4.2.1 Sample collection

Although described in the STAMPEDE protocol since 2006, sample retrieval had not been initiated, necessitating establishment of new processes, collaborations and contractual relationships. I coordinated a call for collaborators to select two trial biobanks. WCB, one of the two selected, received all samples contributing to these analyses. I contacted NHS sites to update site contracts to permit material transfer and reimbursement. Through querying

the trial databases, I generated sample lists which were sent with site guidance in order to request the required samples.

4.2.2 Sample selection

Sampling was limited to participants randomised since November 2011 to ensure that prevalence estimates were generalisable to all metastatic patients. Prior to this date a histological diagnosis was not required by the STAMPEDE protocol in cases of clinically metastatic disease with bone involvement and PSA>100ng/mL. Sample requests were sent to the randomising site and the most tumour rich FFPE tumour blocks containing the PCa samples obtained prior to randomisation were requested i.e. pre-treatment.

The key selection criteria were:

- Metastatic disease at trial entry
- Consented to gift remaining tumour samples
- Randomised from November 2011 onwards at a site participating in sample retrieval

Pathology review was undertaken by a single uro-pathologist (Dr David Griffiths) who assessed tumour content based on a representative Haemotoxylin and Eosin (H&E) stained slide prepared at the WCB if not provided by the site. For analysis two, conducted in collaboration with Almac, an H&E slide was marked with the estimated tumour content. In both analyses, samples with an estimated tumour content of >20% were submitted for sequencing.

4.2.3 Nucleic acid extraction and sequencing

Sequencing analysis was undertaken by two commercial providers. Analysis one was conducted as an extension of an existing collaboration between Clovis Oncology and Foundation Medicine (FM) who supported biomarker screening for the ARIEL programme and subsequent successful FDA submission. Reflecting this rapidly developing technological area and considering the logistics and cost implications of using a provider based in the US, preliminary data was acquired using a second UK-based provider, Almac. Both providers undertook nucleic acid extraction and sequencing using a tNGS panel.

Analysis One: FoundationOne® provided by Foundation Medicine

FoundationOne® (F1) is a clinically accredited tNGS-based test available from FM, a commercial laboratory which is Clinical Laboratory Improvement Amendments (CLIA) certified and College of American Pathologists (CAP) accredited. F1 has been commercialised as a comprehensive cancer genome profiling suitable for all solid tumours[285]. The F1 assay has been validated in FFPE samples where the requirements are: surface areas of ≥25mm^2, nucleated cellularity of ≥80% or ≥30,000 cells and tumour content ≥20%. Library preparation is performed from DNA extracted from unstained FFPE sections and requires a minimum of 50ng dsDNA. The selected areas of interest are amplified by hybridization capture using biotinylated DNA oligonucleotides complementary to 315 exons of cancer-related genes and selected introns where cancer related rearrangements frequently occur. Sequencing is performed using the Illumina HiSeq platform. The typical median coverage is 500X; with >100X at >99% of exons. Four classes of somatic genomic alterations are reported: base substitutions, insertions and deletions (also termed indels), copy number alterations and rearrangements.

The published validation and experience from the first 2221 sequenced samples (mixed solid tumours) reported >99% positive predictive value where the threshold of ≥20% of nucleated cells are tumour derived was met. As part of the validation the concordance with aberrations detected by fluorescent *in-situ* hybridisation (FISH) or IHC was assessed; this included 25 PCa samples with AR-amplification and 22 PCa samples with PTEN deletion; concordance was 100% and 95% respectively. See **A7:** Figure 41 in **Appendix A** for the list of 326 genes covered by F1.

Analysis Two: Ilumina TruSight Tumour T170 Panel provided by Almac Diagnostics

Illumina TruSight Tumour 170 (t170) Panel is a tNGS assay, provided by Illumina and analytically validated by Almac, a commercial provider of companion diagnostics. The t170 has been developed to target both RNA and DNA extracted from FFPE tumour samples. Parallel DNA and RNA library preparation occurs, each requiring 40ng DNA/RNA input. The assay detects base substitutions, insertions and deletions, fusions and splice variants. This project was conducted whilst Almac were providing this as a RUO assay. Analytical validation was ongoing and as such the assay underwent refinement during the conduct of the project. Almac aim to commercialise the t170 as a CLIA compliant assay for prospective use in clinical trials of molecularly-directed therapies, with pharmaceutical companies providing a list of mutations of interest that will be used to define eligibility. Almac incorporate this into the analysis algorithm and report a proportion of the t170 assay

according to the agreed parameters required for the trial. Almac has successfully collaborated with several other clinical trials, including the European Organisation for the Research and Treatment of Cancer (EORTC) SPECTRA programme (Screening Patients for Effective Clinical Trial Access), a pan-European network that aims to support numerous precision medicine trials. See **A7:** Figure 42 in **Appendix A** for the list of 170 genes sequenced as part of the t170. However in contrast to F1, only the proportion of pre-specified genes relevant to the eligibility call are reported, in this case 14 genes involved in homologous recombination. These were defined by Clovis, the pharmaceutical partner and manufacturer of rucaparib, informed by pre-clinical and clinical data.

4.2.4 Assay comparison

Comparison of sequencing methods

Details of the sequencing methods are shown **Appendix A6.** Notable differences include dual DNA and RNA extraction performed by Almac who also routinely macrodissect all samples, aiming to enrich tumour content. Both providers use Illumina sequencing platforms, however the hybrid capture and amplification differ and the comparable coverage at positions relevant to HRD eligibility calls is not known. F1 is a broader assay covering over 300 genes and has been validated to detect copy number alternations. T170 covers 170 genes but excludes intronic regions, which prevents the detection of intronic deletions or intragenic splice site variants that occur across exonic-intronic regions. Additionally, the t170 cannot reliably detect copy number alterations as these failed to meet with pre-defined analytical validation criteria. Also, splice variants, which are called from the RNA component in the t170 assay, are yet to be analytically validated so can only be reported on a RUO basis.

Concordance assessment

20 samples were analysed by both providers in order to assess concordance. Samples underwent DNA extraction by FM and initial sequencing using the F1 assay. Remaining DNA was then transferred to Almac who performed sequencing using the t170 assay. This allowed comparison of the DNA component of both assays across the 126 genes covered by both, see **Appendix 7** for detailed gene lists.

4.2.5 Site pathology survey

All 15 hospital sites contributing samples to analysis one were surveyed to assess pathology practices. Research teams were requested to obtain pathology input when providing

answers to the nine questions listed in **Box 1.** The responses were compared with FM's optimum sample processing guidance, see **Appendix H.**

Box 1: Pathology questionnaire

1. What fixative solution is used?
2. What is the minimum fixation time for prostate biopsies?
3. What is the maximum fixation time?
4. Do you process on a Saturday?
5. Are Friday samples in fixative until Monday?
6. Do you use microwave processing?
7. Do you use xylene free processing?
8. For prostate biopsies, do you use a 'routine' overnight cycle or do you have a specific biopsy processing cycle?
9. If you have a specific biopsy cycle, what is the cycle length?

4.2.6 Biomarker-screening pilot

I designed and wrote an amendment to the STAMPEDE protocol in order to pilot prospective biomarker-screening at a selection of hospital sites, see **Appendix C** for links to online protocol. This will assess the feasibility of enrolment based on HRD, defined as a pathogenic mutation in one or more of 14 HRD genes. This will pilot rapid sample retrieval from a range of sites (district general and large tertiary referral centres) and provide preliminary data on turnaround time. Data from other ongoing STAMPEDE sub-studies has demonstrated that it is feasible to sequence nucleic acid extracted from saliva, FFPE and Streck tubes™ from which circulating tumour DNA (ctDNA) can be isolated. All three sampling approaches will be evaluated as a means to prospective genomic characterisation, with comparative results anticipated in summer 2018. Accruing germline data is presented obtained through saliva sampling analysed by Color Genomics as a preliminary indication of germline prevalence in this population. See **A7:** Figure 43 in **Appendix A7** for the list of genes covered by this germline assay.

4.2.7 Correlative clinical information and data interpretation

Baseline clinical information collected at randomisation was obtained for all participants sampled and the comparable randomised population during the time period (November

2011 to May 2017). Baseline data included all prognostic characteristics such as disease stage, baseline age and performance status, Gleason sum score and presenting PSA. When comparing groups, the Kruskal Wallis non-parametric test was used and a p value <0.05 was considered statistically significant. I performed all analyses using STATA version 15.0 (StataCorp LP, College Station, TX, USA) and additional data management and analysis was undertaken in Microsoft Excel 2010. Supported by Fiona Ingleby, statistician at the MRC CTU at UCL, I generated Venn diagrams to visualise overlap between molecularly defined subsets. Publically available genomic dataset available via cBioPortal (http://www.cbioportal.org) an online resource hosted by Memorial Sloan Kettering Cancer Centre (MSK) were interrogated in order to contextualise these data with other published sequencing studies.

4.3 Results

4.3.1 Population characteristics

Samples from 187 patients, randomised to 1 of 7 research arms between July 2010 and April 2017 were submitted for sequencing as part of analysis one. See **Appendix A8:** Table **73** for all 15 contributing sites and **Appendix A9: Table 75** for the distribution of participants by allocated trial arm**.**

Samples from 100 patients randomised to 1 of 3 arms open between Jul-2014 and May-2017 were submitted for sequencing as part of analysis two. These samples were collected from 28 sites; see **Appendix A8: Table 74** for a site list. **Appendix A9: Table 76** shows the distribution by allocated trial arm.

Both sampled populations were representative of the population enrolled into the STAMPEDE trial. As shown in **Table 47** and **Table 48**, both are comparable with the intention-to-treat (ITT) population, defined as all patients randomised into STAMPEDE during the respective time period. No difference in baseline characteristics were noted between the participants in whom sequencing failed compared with those for whom data was successfully obtained. Therefore the missing sequencing data is assumed to be missing at random.

The patient population identified to have an HRD cancer did not appear significantly different when compared with the total sampled population. There was little evidence to

support a prior hypothesis that HRD cancers may be more prevalent in younger men. Instead, a borderline statistically significant trend was observed for HRD cancers to be observed in older men (Kruskal Wallis test p=0.0421), see **Table 49.**

Table 47: Comparative baseline characteristics (Analysis One: F1)

Baseline Characteristics		Comparative ITT*	Sampled population	Sequenced	Test failed
		N=1043	n=187	n=115	n=72
Age	**Median**	68 years	69 years	70 years	67 years
	Range	40-86 years	44-85 years	44-85	56-80
Presenting PSA (ng/ml	**Median**	97	129	90	149
	IQR	31-338	27-421	17-400	51-455
T stage	**≤T2**	130 (12%)	13 (7%)	9 (8%)	4 (5%)
	T3	590 (57%)	103 (55%)	62 (54%)	41 (57%)
	T4	257 (25%)	59 (32%)	35 (30%)	24 (33%)
	Tx	66 (6%)	12 (6%)	9 (8%)	3 (4%)
Nodal state	**N0**	373 (36%)	72 (38%)	35 (30%)	37 (51%)
	N1	602 (58%)	103 (55%)	72 (63%)	31 (43%)
	Nx	68 (7%)	12 (6%)	8 (7%)	4 (6%)
Disease category	***De novo* M1**	1005 (96%)	183 (98%)	112 (97%)	71 (99%)
	Relapsed M1	38 (4%)	4 (2%)	3 (3%)	1 (1%)
Metastatic distribution	**Bone only**	694 (67%)	127 (68%)	73 (63%)	54 (75%)
	Distant node only	104 (10%)	16 (9%)	12 (10%)	4 (6%)
	Bone & nodal or other	245 (23%)	44 (24%)	30 (26%)	14 (19%)
Gleason	**≤7**	200 (19%)	41 (22%)	20 (17%)	21 (29%)
	8-10	778 (75%)	139 (74%)	91 (79%)	48 (67%)
	Unknown	65 (6%)	7 (4%)	4 (3%)	3 (4%)
Performance status	**0**	757 (73%)	138 (74%)	80 (70%)	58 (81%)
	1-2	286 (27%)	48 (26%)	35 (30%)	14 (19%)

* Comparative ITT population had metastatic disease (M1) at trial entry, randomised during the same time period at the 15 sites who contributed samples to this analysis

Table 48: Comparative baseline characteristics (Analysis two: t170)

Baseline Characteristics		Comparative ITT*	Sampled population	Sequenced	Test failed
		n=773	n=100	n=77	n=23
Age	**Median**	68 years	67 years	68 years	66 years
	Range	37-87 years	45- 85 years	50-85 years	(61-71 years)
Presenting PSA (ng/ml	**Median**	101	112	97	131
	IQR	29-347	43-335	42-372	47-312
T stage	**≤T2**	74 (10%)	14 (14%)	10 (13%)	4 (17%)
	T3	447 (58%)	60 (60%)	46 (60%)	14 (61%)
	T4	175 (23%)	20 (20%)	17 (22%)	3 (13%)
	Tx	77 (10%	6 (6%)	4 (5%)	2 (9%)
Nodal state	**N0**	256 (33%)	32 (32%)	27 (35%)	5 (22%)
	N1	479 (62%)	61 (61%)	45 (58%)	16 (70%)
	Nx	38 (5%)	7 (7%)	5 (6%)	2 (9%)
Disease category	***De novo* M1**	747 (97%)	100 (100%)	77 (100%)	23 (100%)
	Relapsed M1	26 (3%)	0	0	0
Metastatic distribution	**Bone only**	498 (64%)	65 (65%)	47 (61%)	18 (78%)
	Distant node only	86 (11%)	11 (11%)	9 (12%)	2 (9%)
	Bone & nodal or other	189 (24%)	24 (24%)	21 (27%)	3 (13%)
Gleason	**≤7**	132 (17%)	9 (9%)	8 (10%)	1 (4%)
	8-10	537 (69%)	84 (84%)	62 (81%)	22 (96%)
	Unknown	104 (13%)	7 (7%)	7 (9%)	0
Performance status	**0**	538 (70%)	70 (70%)	56 (73%)	14 (61%)
	1-2	235 (30%)	30 (30%)	21 (27%)	9 (39%)

* Comparative ITT population had metastatic disease (M1) at trial entry, randomised during the same time period at the 28 sites who contributed samples to this analysis.

Table 49: Characterising the biomarker-positive (HRD) subgroup

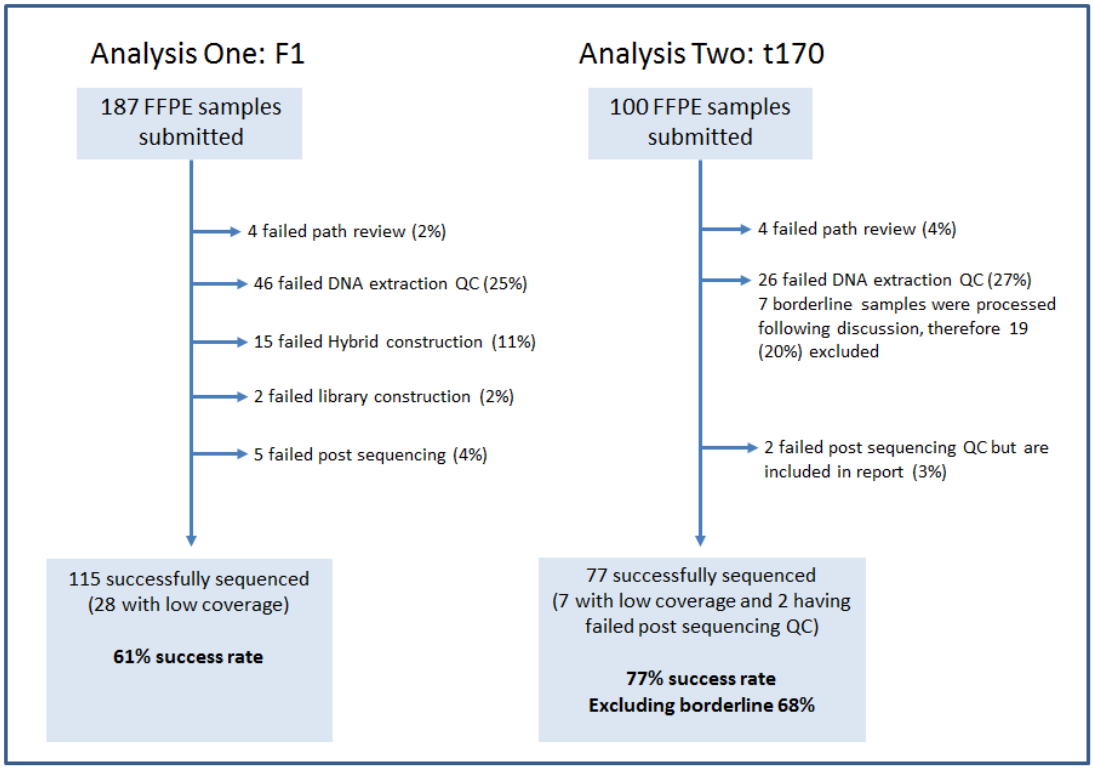
Baseline Characteristics		Total Sampled population	HRD	Biomarker negative	Biomarker unknown	p value*
		n=287	n=28	n=164	n=95	
Age	**Median**	68 years	70 years	69 years	67 years	**0.0421**
	Range	44- 85 years	52-82 years	44-85 years	45-80 years	
Presenting PSA (ng/ml	**Median**	126	138	90	148	0.1461
	IQR	35-400	56- 1179	23-384	48-435	
T stage	**≤T2**	27 (9%)	2 (7%)	17 (10%)	8 (8%)	0.9730
	T3	163 (57%)	16 (57%)	92 (56%)	55 (58%)	
	T4	79 (28%)	9 (32%)	43 (26%)	27 (28%)	
	Tx	18 (6%)	1 (4%)	12 (7%)	5 (5%)	
Nodal state	**N0**	104 (36%)	6 (21%)	56 (34%)	42 (44%)	0.0711
	N1	164 (57%)	19 (68%)	98 (60%)	47 (47%)	
	Nx	19 (7%)	3 (11%)	10 (6%)	6 (6%)	
Disease category	***De novo* M1**	283 (99%)	28 (100%)	161 (98%)	94 (99%)	0.7044
	Relapsed M1	4 (1%)	0	3 (2%)	1 (1%)	
Metastatic distribution	**Bone only**	192 (67%)	17 (61%)	103 (63%)	72 (76%)	0.0770
	Distant node only	27 (9%)	0	21 (13%)	6 (6%)	
	Bone & nodal or other	68 (24%)	11 (39%)	40 (24%)	17 (18%)	
Gleason	**≤7**	50 (17%)	5 (18%)	23 (14%)	22 (28%)	0.0950
	8-10	223 (78%)	18 (64%)	135 (82%)	70 (74%)	
	Unknown	14 (5%)	5 (18%)	6 (4%)	3 (3%)	
Performance status	**0**	208 (72%)	19 (68%)	117 (71%)	72 (76%)	0.6298
	1-2	79 (28%)	9 (32%)	47 (29%)	23 (24%)	

* p value for Kruskal Wallis test for differences between groups, values statistically significant at 5% level shown in bold

4.3.2 Feasibility data

Overall, 192 out of 287 samples (67%) were successfully sequenced. The sequencing success rate was 61% and 77% in analysis one and two respectively. In seven cases sequenced as part of analysis two the minimum DNA concentration of 3.3ng/µL was just missed. In 5/7 cases results could be reported, although with reduced confidence and copy number alterations were not reliable. The most frequent reason for failure in both analyses was inadequate concentration of sufficiently high quality DNA, resulting in quality control (QC) failure post DNA extraction.

Figure 33: Sequencing success rate



Site variability

As shown in **Table 50**, the sequencing success rate also varied by hospital site. In total, samples from 287 patients randomised at 35 sites were included, with between 1-47 patients sampled per site. Sequencing success rates are judged preliminary where <5 patients had been sampled (highlighted in grey). In the 9 sites where >10 patients have been sampled the sequencing success rate ranges from 21-100%.

Table 50: Sequencing success by site (both analyses combined)

Site name	Site number	Number of cases sampled per site	Successfully sequenced	Success rate (%)
Velindre, Cardiff	2	47	36	77%
Singleton, Swansea	11	38	17	45%
Stepping Hill, Stockport	41	21	6	29%
Royal United Hosp Bath	72	19	4	21%
Ipswich	39	15	14	93%
Broomfield Hosp	32	13	13	100%
Huddersfield	40	13	10	77%
Royal Devon & Exeter	7	13	11	85%
Torbay DGH	20	11	11	100%
Bristol	24	9	6	67%
Pilgrim Hosp	114	7	5	71%
Raigmore Hosp	57	7	6	86%
York Hosp	133	7	2	29%
Burnley General Hosp	59	6	6	100%
Guy's Hosp, London	13	6	5	83%
Barnet General Hosp	115	5	3	60%
Darlington Memorial Hosp	53	5	2	40%
Lister Hosp	55	5	0	0%
Lincoln County Hosp	113	4	4	100%
Royal Albert Edward Infirmary	65	4	3	75%
Royal Bolton Hosp	56	4	2	50%
Musgrove Park Hosp	51	3	2	67%
Royal Bournemouth Hosp	22	3	2	67%
University College Hosp	38	3	3	100%
Weston General Hosp	112	3	2	67%
King's Mill	102	2	2	100%
North Middlesex Hosp	46	2	2	100%
Queen Alexandra, Portsmouth	75	2	1	50%
Southend University Hosp	31	2	1	50%
Worthing Hosp	43	2	2	100%
Essex County Hosp	124	1	0	0%
Hereford	42	1	0	0%
James Cook University Hosp	28	1	1	100%
Southampton General Hosp	17	1	1	100%
Yeovil District Hosp	128	1	1	100%

	≥5 samples and above average
	≥5 sample and below average
	<5 samples; data preliminary

Exploring pre-analytical variables

The site variability was higher than anticipated and so in order to explore potential explanations for this a survey of fixation protocols was conducted, comparing reported practices against those recommended by FM (see **Appendix H**). Responses were received from 14/15 sites contributing samples for analysis one, summarised in **Table 51-Table 53.**

Table 51: Sample numbers and success rate by site (analysis one)

Site name	Site number	Sample number	Successfully sequenced	Success rate (%)
Broomfield Hosp, Chelmsfor	32	11	11	100%
Raigmore, Inverness	57	5	5	100%
Ipswich Hosp	39	13	12	92%
Royal Devon & Exeter	7	7	6	86%
Huddersfield	40	13	10	77%
Velindre, Cardiff	2	42	32	76%
Bristol	24	9	6	67%
Singleton, Swansea	11	36	16	44%
Stepping Hill, Stockport	41	21	6	29%
Royal United Hosp Bath	72	19	4	21%
Blackburn, East Lanc	59	3	3	100%
King's Mill	102	2	2	100%
Hereford	42	1	1	100%
Queen Alexandra, Portsmou	75	2	1	50%
Bolton	56	2	1	50%

Table 52: Minimum and maximum fixation times at sites participating in analysis one

		Minimum fixation time				Maximum fixation time					
Site number	Success rate (%)	0-4hrs	4-8hrs	8-12hrs	>12hrs	0-6hrs	12-24hrs	24-36hrs	36-48hrs	48-72hrs	>72hrs
32	100%		X						X		
57	100%	X								X	
39	92%			X					X		
7	86%		X				X				
40	77%		X					X			
2	76%	X					X				
24	67%	*missing*									
11	44%		X							X	
41	29%			X						X	
72	21%		X							X	
59	100%				X					X	
102	100%				X					X	
42	100%				X						X
75	50%		X			X					
56	50%		X					X			

KEY	
	Low performing site
	<4 samples; limited information
	Optimum process
	Not recommended

Table 53: Weekend processing at sites participating in analysis one

		Weekend processing	
Site number	Success rate (%)	Saturday processing?	Are Friday samples in fixative until Monday?
32	100%	No	Only if received after 16:30 (very rare)
57	100%	No	Yes
39	92%	Yes	No
7	86%	Yes	No
40	77%	No	No
2	76%	No	Yes
24	67%	missing data	
11	44%	No	No
41	29%	No	Yes
72	21%	No	No
59	100%	No	Yes
102	100%	No	Yes
42	100%	No	Yes
75	50%	No	No
56	50%	No	No

KEY	
	Low performing site
	<4 samples; limited information
	Optimum process
	Not recommended

Pathology Survey results

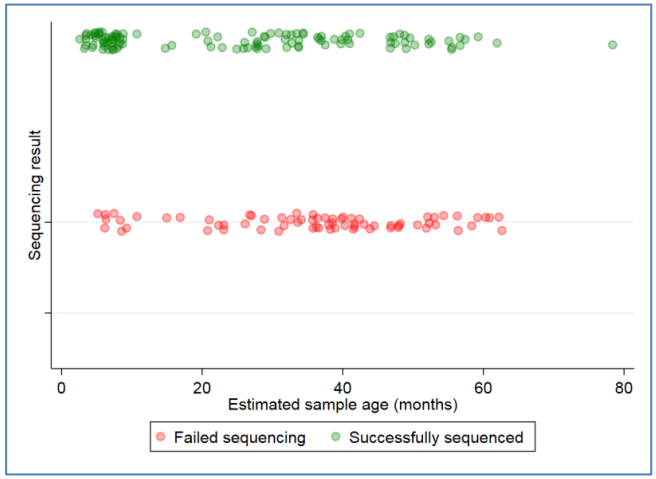
All sites were using the recommended fixation solution (10% neutral-buffered formalin) and 9 out of 13 were within the recommended fixation time of 6-72 hours. Numbers are too small to permit statistical analyses but it is notable that longer fixation times were observed in the lower performing sites (site numbers 11, 42, 72). 3 sites reported shorter than the recommended fixation times, although this is thought to have less impact on DNA quality. 5 sites reported samples received on Friday could remain in fixative over the weekend, thus exceeding the maximum recommended time; however this was not observed to associate with sequencing success rates. The length of cycle is also an indication of how long samples will remain in fixative and exposed to other agents and processes that may impair nucleic acid integrity. In all of the 3 low performing sites a routine biopsy cycle, which is typically longer than a biopsy specific cycle, was used, although site 72 reported a rapid biopsy programme was used for samples received before 11.45am. All but one of the good

performing sites reported using a shortened biopsy cycle. No protocol included xylene free or microwave processing.

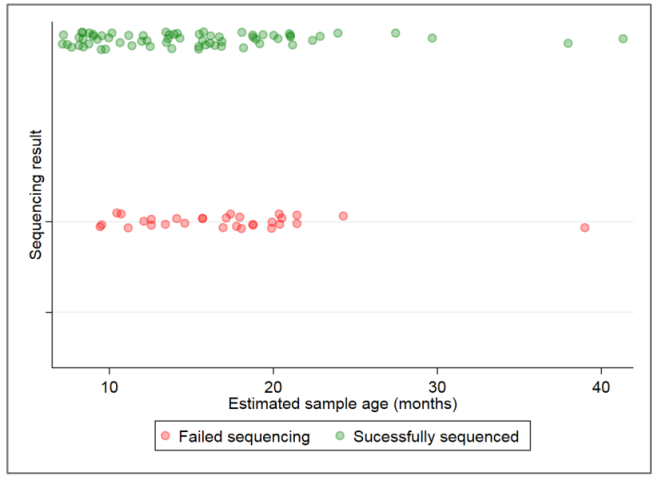
Exploring sample age

The impact of sample age was first assessed in analysis one (**Graph 14**) and suggested a higher success rate in younger samples. Based on this finding, the selection criteria for analysis two were changed to include more samples estimated to be less than 12 months old. A further assessment of the impact of age within analysis two is shown in **Graph 15,** and suggests that the relationship is less clear. Younger samples were selected for analysis two however there was a range and high sequencing success was observed in all regardless of age. One of the challenges in the assessment of sample age is that it is likely a surrogate for other factors e.g. if a site fixation practice changed during this time. As shown in **Graph 16**, the sites with low success rates (11, 41, and 72) contributed a high number of older samples which likely confounds the assessment of age. In some higher performing sites such as 7, 32, and 39, DNA yield and therefore sequencing success appears independent of sample age.

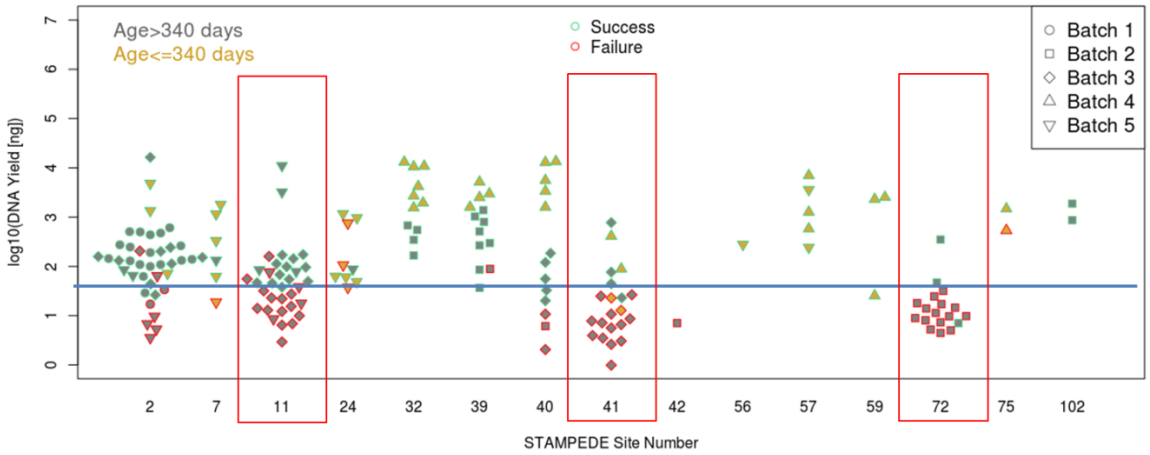
Graph 14: Success rate by sample age (analysis one: F1)



Graph 15: Success rate by sample age (analysis two: t170)



Graph 16: Success rate by sample age, batch and site (analysis one)



Exploring the impact of sample age on sequencing success rate: Graph 14 suggests that FFPE samples <20 months old are more likely to yield sufficient high quality nucleic acids required for targeted next-generation sequencing (tNGS). However the relationship is less clear in Graph 15 which summarises the results of analysis two. The samples selected for this analysis were on average younger and overall a higher success rate was observed for this assay. Graph 16 shows that the three sites with the highest failure rates contributed a large number of older samples, likely confounding the analysis of sample age.

4.3.3 Prevalence data

The results of analysis one demonstrated 16/115 cases harboured a mutation in an HRD gene, equivalent to a prevalence of 14% (95% CI 9-19%). *BRCA2* mutations were reported in 2/115 (2%), but both *ATM* and *CDK12* mutations were more frequent, each occurring in 6%. No *BRCA1* mutations were detected. Based on the biomarker strategy adopted in the evaluation of rucaparib in mCRPC, 8% would be eligible for the *BRCA1*, *BRCA2* or *ATM* mutant group. These findings are consistent with the results of analysis two (as reported in May-2018). 12/77 cases were found to have one or more mutations in a HRD gene, equivalent to a prevalence of 16% (95% CI 9-23%). *BRCA2* mutations were identified in 3% and *ATM* mutations in 10%. When the populations are combined, the estimated HRD prevalence is 15% (95% 11-19%); which includes *ATM* (8%), *CDK12* (5%) and *BRCA2* (2%). *BRCA1* mutations are not observed.

Table 54: Summary of HRD prevalence data

	Analysis One (F1)		Analysis Two (t170)		Combined	
HRD Gene	**n**	**%**	**n**	**%**	**n**	**%**
ATM	7	6%	8	10%	15	8%
BARD1	0	0%	0	0%	0	0%
BRCA1	0	0%	0	0%	0	0%
BRCA2	2	2%	2	3%	4	2%
BRIP1	0	0%	0	0%	0	0%
CDK12	7	6%	3	4%	10	5%
CHEK2	0	0%	1	1%	1	1%
NBN	0	0%	0	0%	0	0%
PALB2	0	0%	0	0%	0	0%
Rad51	0	0%	0	0%	0	0%
Rad51B	0	0%	0	0%	0	0%
Rad51C	0	0%	0	0%	0	0%
Rad51D	0	0%	0	0%	0	0%
Rad54L	0	0%	0	0%	0	0%
Total number of mutations	16		14		30	
Number of pts with a mutation	16		12		28	
Total pts sequenced	115		77		192	
Prevalence (%)	14%		16%		15%	
	(95% CI 9-19%)		(95% CI 9-23%)		(95% CI 11-19%)	

Table 55: Analysis One (F1) sample level prevalence data

Pt #	Gene	Event Type	Event Detail	Position
1	ATM	short-variant	1564_1565delGA	chr11:108121755
2	ATM	short-variant	7921C>T	chr11:108203621
3	ATM	short-variant	3497_3498insC	chr11:108151816
4	ATM	short-variant	3228delA	chr11:108143522
4	ATM	copy-number-alteration (loss)	-	-
5	ATM	short-variant	8786+1G>A	chr11:108224608
6	ATM	short-variant	7195C>T	chr11:108199853
7	BRCA2	short-variant	2588_2588delA	chr13:32911079
8	BRCA2	short-variant	5722_5723delCT	chr13:32914213
8	CDK12	short-variant	3142C>T	chr17:37680973
10	CDK12	short-variant	2880G>A	chr17:37673726
11	CDK12	short-variant	1025_1026insT	chr17:37619349
12	CDK12	short-variant	4382_4383insG	chr17:37687478
13	CDK12	short-variant	2520delG	chr17:37657602
14	CDK12	short-variant	304_314delTCAGATCGGAG	chr17:37618627
15	CDK12	short-variant	2126delA	chr17:37649020

Table 56: Analysis Two (t170) sample level prevalence data

Pt #	Gene	Event Type	Event Detail
1	CDK12	short-variant	ENST00000447079.4:c.2670_2673delCCCT
2	ATM	short-variant	ENST00000278616.4:c.323delC
	ATM	short-variant	ENST00000278616.4:c.1355delC
3	BRCA2	short-variant	ENST00000544455.1:c.4936_4939delGAAA
4	ATM	short-variant	ENST00000278616.4:c.3802delG
5	ATM	short-variant	ENST00000278616.4:c.481C>T
6	CHEK2	short-variant	ENST00000382580.2:c.283C>T
7	ATM	short-variant	ENST00000278616.4:c.7939dupA
8	BRCA2	short-variant	ENST00000544455.1:c.5303_5304delTT
9	ATM	short-variant	ENST00000278616.4:c.3165T>G
10	ATM	short-variant	ENST00000278616.4:c.4875_4876insCGGA
11	CDK12	short-variant	ENST00000447079.4:c.366delA
	CDK12	short-variant	ENST00000447079.4:c.452delC
12	ATM	short-variant	ENST00000278616.4:c.972_973insG

Comparing approaches adopted in analysis one and two

The results of the concordance analysis between analysis one and two demonstrated a high level of agreement. 20 samples were processed (DNA extraction and library preparation) and sequenced by FM and remaining extracted DNA was subsequently analysed by Almac who were blind to the prior results. 17/ 20 samples passed DNA QC and were successfully sequenced. Results were concordant in 16/17 cases including 7/8 cases with a pathogenic HRD aberration as reported in analysis one. The only discordant result was for #7, *ATM* copy number loss, detected in analysis one but not in analysis two.

Table 57: Comparative HRD results (n=20)

				Analysis 1 (FM)	Analysis 2 (Almac)
#	patID	HRD Gene	c_cds		
1	900010	CDK12	3142C>T	HRD +	Test failure
2	900012	CDK12	2880G>A	HRD +	HRD+
3	900021	BRCA2	2588_2588delA	HRD+	HRD+
4	900117			Not HRD+	Not HRD+
5	900120	ATM	1564_1565delGA	HRD+	HRD+
6	900132	ATM	7921C>T	HRD+	HRD+
7	900206	ATM (loss)	-	HRD+	Not HRD+
8	900270			Not HRD+	Not HRD+
9	900356			Not HRD+	Not HRD+
10	900357			Not HRD+	Not HRD+
11	900366			Not HRD+	Not HRD+
12	900368			Not HRD+	Not HRD+
13	900369	BRCA2	6469C>T	HRD+	HRD+
		BRCA2	5722_5723delCT		
14	900371	CDK12	1414delG	HRD+	
		CDK12	2520delG		
15	900372	CDK12	304_314delTCAGATCGGAG	HRD+	HRD+
16	900375			Not HRD+	Not HRD+
17	900395			Not HRD+	Not HRD+
18	900398			Not HRD+	Not HRD+
19	900481			Not HRD+	test failure
20	900483	CDK12	2126delA	HRD+	test failure

Key: The only HRD discordant case is highlighted in pink

Exploring the impact of the bioinformatic algorithm (Analysis two: t170)

The RUO results of the t170 assay were refined during the course of this study. All samples were sequenced once by Almac in randomised batches, however several updates were required to the bioinformatic interpretation of the sequencing data. The first update was required to include all mutations predicted to result in frameshift variants resulting in a premature stop codon. This type of variant is predicted to be functionally significant. Subsequently, two further updates were required to refine the data and impose sufficient QC filters to exclude low confidence variant calls and suspected FFPE artefacts. As shown by **Table 58**, updating the bioinformatic interpretation to exclude suspected artefacts had the greatest impact on the overall prevalence rate, which as of May-2018, is now in line with the estimate obtained by the fully-validated F1 assay.

Table 58: Impact of revised bioinformative algorithm (Analysis two: t170)

	Jan-18		Feb-18		Apr-18		May-18	
HRD gene	n	%	n	%	n	%	n	%
ATM	5	6%	16	21%	10	13%	8	10%
BARD1	1	1%	9	12%	6	8%	0	0%
BRCA1	1	1%	6	8%	2	3%	0	0%
BRCA2	10	13%	17	22%	6	8%	2	3%
BRIP1	0	0%	3	4%	2	3%	0	0%
CDK12	1	1%	10	13%	5	6%	3	4%
CHEK2	2	3%	3	4%	2	3%	1	1%
NBN	3	4%	4	5%	0	0%	0	0%
PALB2	3	4%	3	4%	0	0%	0	0%
RAD51	0	0%	0	0%	0	0%	0	0%
RAD51B	2	3%	2	3%	0	0%	0	0%
RAD51C	1	1%	1	1%	0	0%	0	0%
RAD51D	0	0%	0	0%	0	0%	0	0%
RAD54L	0	0%	0	0%	0	0%	0	0%
Total number of mutations	29		74		50		14	
Number of pts with a mutation	10		27		23		12	
Total pts sequenced	77		77		77		77	
Prevalence (%)	13%		35%		30%		16%	
	(95% CI 7-19%)		(95% CI 26-44%)		(95% CI 21-39%)		(95% CI 9-23%)	

Updates to Bioinformative algorithm	**Update:** Include frameshift variants resulting in a premature stop codon	**Update:** Application of revised QC filters to remove low-confident calls	**Update:** Application of further revised filters to exclude suspected FFPE artefacts (significant cause of false positives)

4.3.4 Germline prevalence

Preliminary data is available from the ongoing biomarker screening pilot to inform the prevalence of germline HRD in men presenting with metastatic castrate-sensitive prostate cancer. As of May-2018, 40 patients have undergone germline analysis see **Table 59.** Two pathogenic aberrations have been detected, one well-established germline pathogenic duplication in *BRCA2* occurring at location 13q13.1 which results in a frameshift, see #8[286] and a second heterozygous *CHEK2* mutation, see #25. The germline HRD prevalence is therefore currently estimated at 5% of which *BRCA2* is 2.5% (95% CI -2 to 7%). No other pathogenic mutations were reported in this assay that covers 10 HRD genes of interest; see **Appendix A7: Figure 43.**

Table 59: Preliminary germline prevalence data

#	Gene	Type	cHGVS	Exon	Transcript	Zygosity	Classification
1	APC	SNV	c.7472T>C	16	ENST00000257430	Heterozygous	VUS
2	BRIP1	SNV	c.2233G>A	15	ENST00000259008	Heterozygous	VUS
3							no VUS/LP/P detected
4							no VUS/LP/P detected
5							no VUS/LP/P detected
6							no VUS/LP/P detected
7							no VUS/LP/P detected
8	BRCA2	indel	c.1813dupA	10	ENST00000544455	Heterozygous	Pathogenic
9	BARD1	SNV	c.2123A>G	11	ENST00000260947	Heterozygous	VUS
	PMS2	SNV	c.632G>A	6	ENST00000265849	Heterozygous	VUS
10							no VUS/LP/P detected
11	ATM	SNV	c.1650A>G	11	ENST00000278616	Heterozygous	VUS
12							no VUS/LP/P detected
13							no VUS/LP/P detected
14	BRCA2	SNV	c.1466C>G	10	ENST00000544455	Heterozygous	VUS
15							no VUS/LP/P detected
16	BRCA2	SNV	c.7819A>G	17	ENST00000544455	Heterozygous	VUS
17							no VUS/LP/P detected
18	BAP1	SNV	c.1806G>C	14	ENST00000460680	Heterozygous	VUS
19							no VUS/LP/P detected
20							no VUS/LP/P detected
21							no VUS/LP/P detected
22							no VUS/LP/P detected
23							no VUS/LP/P detected
24							no VUS/LP/P detected
25	CHEK2	indel	c.1100delC	11	ENST00000328354	Heterozygous	Pathogenic
26							no VUS/LP/P detected
27	RAD51D	SNV	c.629C>T	7	ENST00000345365	Heterozygous	VUS
	BRCA2	SNV	c.9302T>G	25	ENST00000544455	Heterozygous	VUS
28	SMAD4	SNV	c.424+5G>A	3	ENST00000342988	Heterozygous	VUS
29	BARD1	SNV	c.965G>A	4	ENST00000260947	Heterozygous	VUS
30							no VUS/LP/P detected
31							no VUS/LP/P detected
32							no VUS/LP/P detected
33							no VUS/LP/P detected
34							no VUS/LP/P detected
35							no VUS/LP/P detected
36							no VUS/LP/P detected
37							no VUS/LP/P detected
38							no VUS/LP/P detected
39	ATM	SNV	c.4148C>T	28	ENST00000278616	Heterozygous	VUS
40							no VUS/LP/P detected

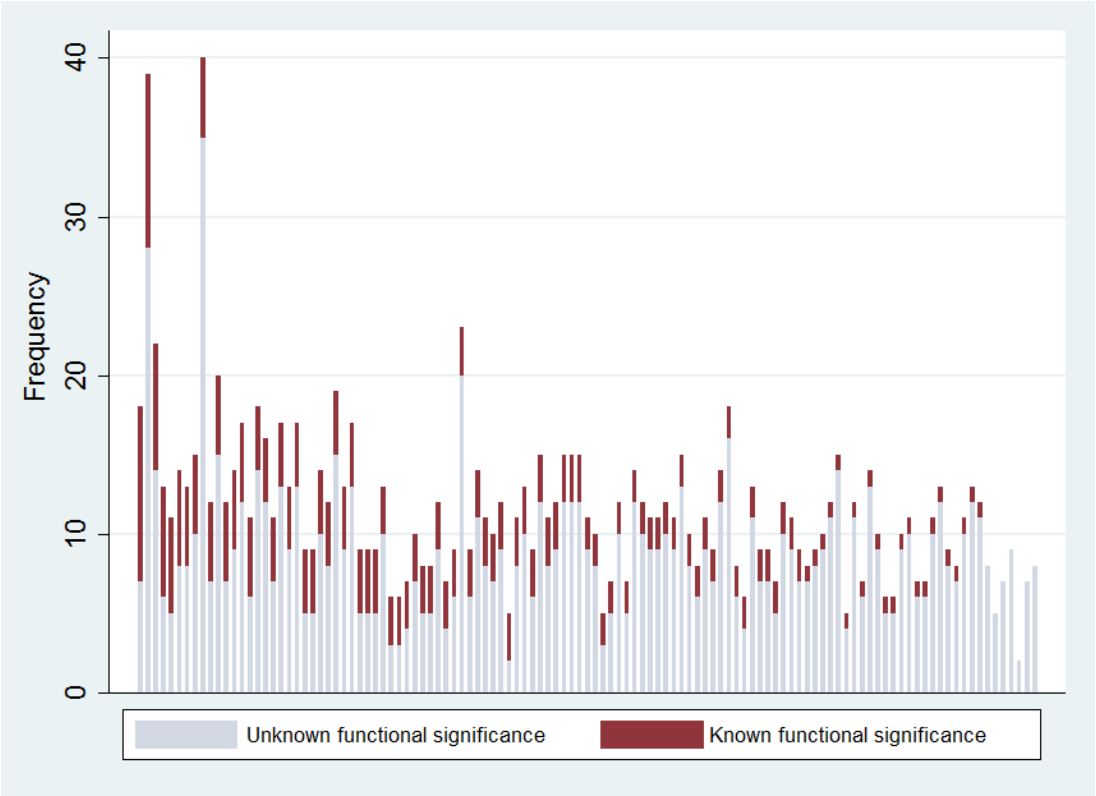
Key: Chr, Chromosome; Ref, Reference; Alt, Alternative; SNV, Single Nucleotide variant; VUS, Variant of Unknown Significance; LP, Likely Pathogenic; P, Pathogenic.

4.3.5 Prevalence of other genomic aberrations

The prevalence of other non-HRD aberrations is shown by the results of analysis one as the full F1 report was provided for all of the 115 successfully sequenced cases. In contrast, the results of analysis two (t170) were limited to the 14 HRD genes of interest so cannot inform on the broader genomic profiles.

In total 1333 mutations were detected of which 24% were reported as likely or known pathogenic. 94% of samples (108/115) harboured one or more pathogenic mutation; the median number per case was 3 (range 1-11), as shown by **Graph 17.** When limited to genes previously reported to be mutated in prostate cancer in published series e.g. SU2C-PCF[39], 315 pathogenic mutations were detected. 83% (96/115) of samples had a pathogenic mutation in one or more of these genes, typical of the 'long-tail' previously described[33].

Graph 17: Mutations per case highlighting those of clinical significance (Analysis One)



Each bar represents a patient successfully sequenced as part of analysis one (F1) n=115. The median number of aberrations reported per case was 11 (range 2-40), however 76% of all reported mutations were variants of unknown significance (VUS) highlighting the "background noise" which either represents normal variation or incomplete knowledge of pathogenic changes.

The most prevalent likely or known pathogenic aberration reported was *PTEN* loss which occurred in 39/115 cases (34%). This was due to copy number loss in 29 cases and insertion, deletion or point mutation in the remaining 10. The second most common aberrations were *TP53* mutations and *TMPRSS2* rearrangements, which occurred in 38 (33%) and 37 (32%) cases respectively. *TP53* is a well characterised gene which is frequently mutated in multiple cancer types and all mutations identified in this cohort are recognised to be likely (n=6) or known (n=32) pathogenic. Within the 47 detected *TMPRSS2* rearrangements, 37 are recognised to be pathogenic and a further 10 are VUS. In contrast with mCRPC, there were no detected *AR* aberrations in this treatment naïve cohort, see **Table 60.**

Table 60: Summary of prevalence data (analysis one)

Summary of significant gene/pathway variants (n=115)		**n**	**%**
AR	*AR*	0	0%
TMPRSS2	*TMPRSS2* rearrangements	37	32%
PTEN	*PTEN* (small variant or copy number loss)	39	34%
TP53	*TP53*	38	33%
PIK3	*AKT1, AKT3, PIK3C2B, PIK3C2G, PIK3C3, PIK3CA, PIK3CB, PIK3CG, PIK3R1, PI3K3R2*	21	18%
RAF	*BRAF, RAF1*	3	3%
Wnt	*APC, CTNNB1, RNF43, ZNRF3*	16	14%
HRD	*ATM, BARD1, BRCA2, BRCA1, BRIP1, CDK12, CHEK2, NBN, RAD50, RAD51, RAD51B, RAD54L*	16	14%
MSI	*MSH2, MSH5, MLH1*	3	3%
Cell cycle	*CDKN1B, CDKN2A, CDKN2B, RB1*	7	6%
Chromatin modifier	*MLL2, KMT2C, MLL3, KDM5C*	12	10%
Other	*SPOP*	5	4%

Pathway analysis was undertaken using the genes reportedly aberrant in CPRC as reported by the SU2C/PCF collaboration in order to generate exploratory comparative prevalence data relevant to this disease setting[39]. PI3K pathway aberrations were common and observed in 18%. As seen in mCRPC, *PI3K* mutations co-existed with *PTEN* deficiency in 9%, see **Figure 37**. The Wnt signalling pathway was aberrant in 14%, with pathogenic mutations in *APC* 7% and *CTNNB1* in a further 7%. Cell-cycle pathway mutations were observed in 10%, with *RB1* mutations present in 3% and mutations in cyclin-dependent kinases e.g. *CDKN1B* present in a further 3%. 14% of the cohort had a predicted pathogenic mutation in one of the 12 HRD genes reported as part of analysis one. In total, 49/115 (43%) had a mutation in one or more HRD genes, but 45% of all mutations in HRD genes were reported as VUS. Pathogenic *SPOP* mutations were detected in 5 cases (4%). Pathogenic *BRAF*

mutations were detected in 3 cases; no pathogenic *RAF1* mutations were reported. Overall 3% had evidence of MSI with pathogenic mutations in *MSH2* or *MSH6*, a further 5 VUS were detected in *MLH1*, *MSH2* and *MSH6*.

4.3.6 Overlap between molecularly-defined groups

Knowledge of the overlap between molecularly-defined groups is required to determine the feasibility of evaluating multiple molecular-directed therapies in one trial platform. Using the results from analysis one, the overlap between pathway aberrations and frequently mutated genes was explored within this cohort (n=115). The observed overlap is presented in two ways: limited to mutations known to be significant and all mutations, regardless of current predictions of pathogenicity in anticipation that this will change as knowledge is acquired.

Overlap observed in Analysis One (F1)

Pathway aberrations were observed to frequently co-exist. Specifically, as shown in **Figure 34** and **Figure 35**, HRD mutations were observed to overlap with *PTEN* loss, *TMPRSS2* rearrangements, cell cycle pathway defects, MSI, Wnt pathway defects and aberrations in chromatin modifiers. The only finding of mutually exclusivity was with *BRAF* mutations which are rare (3%). 68% of all mutations detected in HRD genes were reported as VUS, therefore in all cases, the degree of overlap increases considerably when all mutations are included regardless of predicted clinical significance. As shown in **Figure 36** overlap is also observed between all other pathway aberrations, with the largest overlap seen between *PTEN* loss and PI3K pathway aberrations which co-exist in 9% overall.

PTEN deficiency has been suggested as a predictive biomarker for AKT-inhibitors[287]. In addition PI3K pathway aberrations may predict sensitivity to PI3K-inhibitors, whilst Wnt pathway aberrations have been shown to predict sensitivity to porcupine inhibitors in pre-clinical studies[288]. CDK inhibitors have been shown to be active in other tumours with evidence of cell-cycle pathway aberrations involving *CDKN2A* or *CCND1* as observed in this cohort[289]. Together with HRD pathway defects, these groups represent the most therapeutically significant based on current knowledge. As shown in **Figure 37**, 67 patients have one or more therapeutically relevant mutations, however again these often co-exist. Therefore future trial platforms aiming to co-recruit to multiple molecularly-defined cohorts would require a hierarchical system to determine allocation.

Comparison with publically available datasets

Through interrogation of publically available datasets, available via cBioPortal, there was some evidence that *BRAF-RAF1* mutations are mutually exclusive with HRD in prostate cancer. However in larger datasets this is limited to a subset of HRD genes and these were not consistent. In the MSK-IMPACT cohort *BRAF* mutations were mutually exclusive with mutations in *BRCA1*, *BRIP1*, *CHEK2*, *Rad50*, *Rad51*, *Rad51B* and *Rad54L*[40]. In the SU2C cohort *BRAF* mutations were mutually exclusive with *BARD1*, *BRCA1*, *BRIP1*, *CHEK2* and *Rad51* mutations. In the largest cohort (n=1013), only 3 HRD genes were mutually exclusive with *BRAF* (*FANCA*, *NBN* and *RAD51D*); and 8 HRDs mutually exclusive with *RAF1* (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12*, *Rad51*, *Rad51D*)[39]. Interestingly, no HRD genes were mutually exclusive with *BRAF* or *RAF1* in the MSK-IMPACT pan-cancer cohort (n=10,945). Whilst this most likely represents the power of this very large dataset to detect overlap between rare aberrations, it may also suggest that this is a prostate specific finding[290].

Figure 34: Overlap between HRD mutations and other pathway aberrations -1

Significant mutations	All mutations
Figure 32a	**Figure 32b**

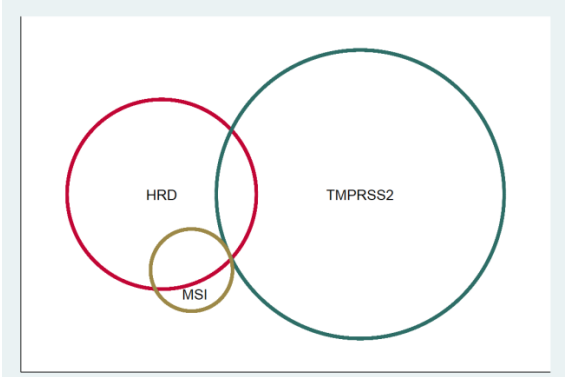
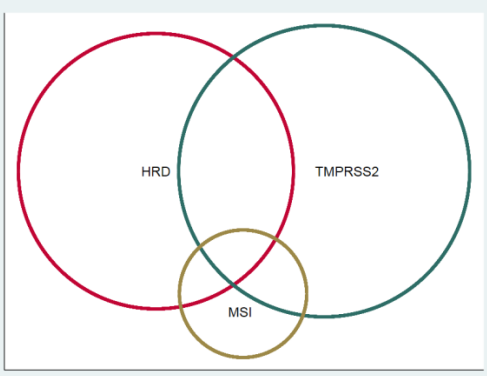
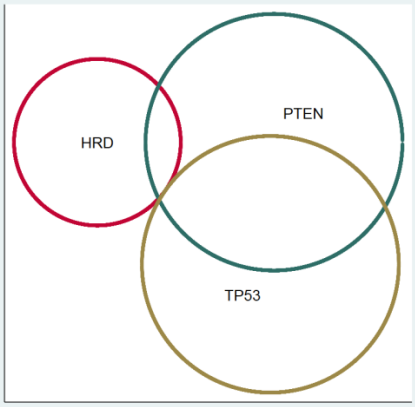
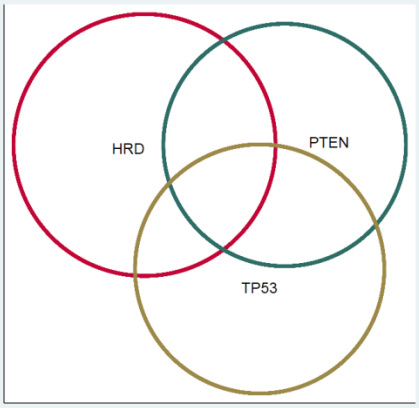

	

Figure 32c	**Figure 32d**
	

Overlap between HRD (red circles) and other mutations of interest: When limited to mutations of known significance 2 out of 3 cancers with MSI also have HRD (Figure 32a) and *TMPRSS2* rearrangements and HRD co-exist in 2 cases (Figure 32c). HRD is mutually exclusive with known significant *TP53* mutations (Figure 32c) but 2 cases have co-existing *PTEN* loss and HRD. When all mutations are included all instances of mutual exclusivity are no longer observed (Figure 32d).

Figure 35: Overlap between HRD mutations and other pathway aberrations -2

Significant mutations	All mutations
Figure 33a	**Figure 33b**

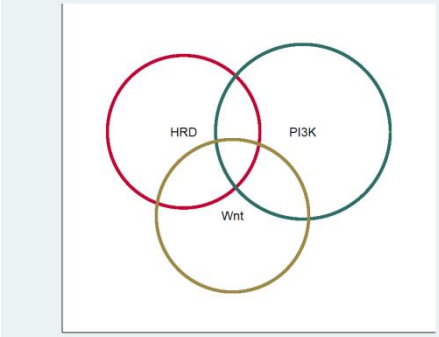
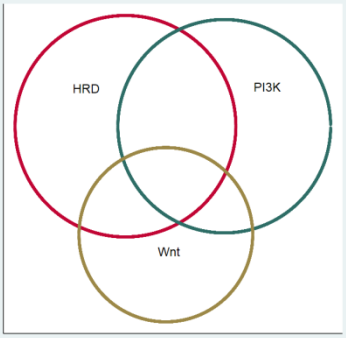
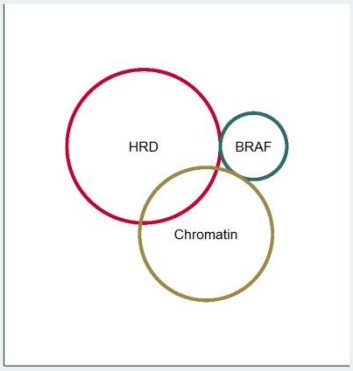
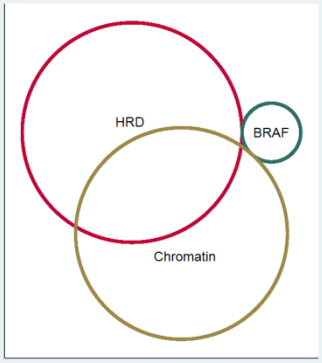

	

Figure 33c	**Figure 33d**
	

Overlap between HRD (red circles) and other mutations of interest: HRD mutations are observed to co-exist with *PIK3* mutations, Wnt pathway aberrations (**Figure 33a**) and mutations in chromatin modifiers (**Figure 33c**). HRD mutations are mutually exclusive with *BRAF* mutations, including when mutations of unknown significance are included (**Figures 33c and 33d**).

Figure 36: Overlap between non-HRD pathway aberrations

Significant mutations	All mutations
Figure 34a	**Figure 34b**

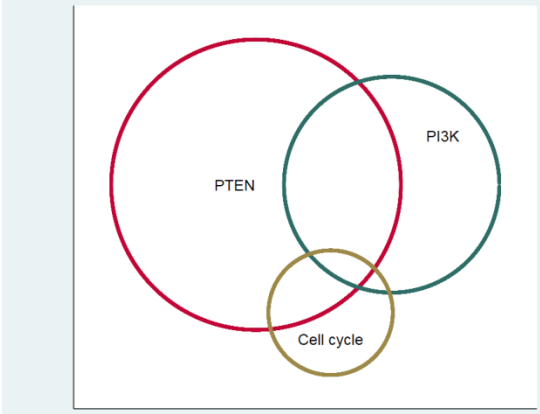
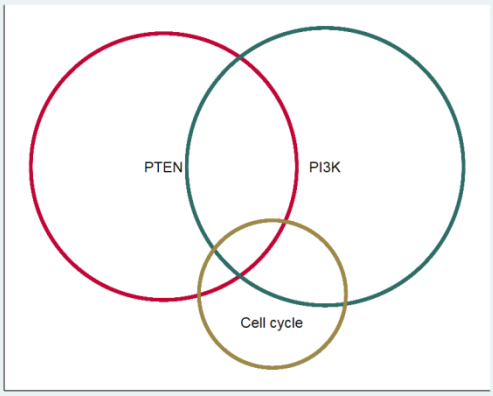

	

Figure 34c	**Figure 34d**

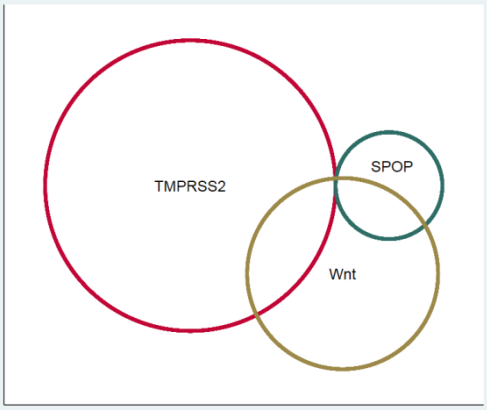
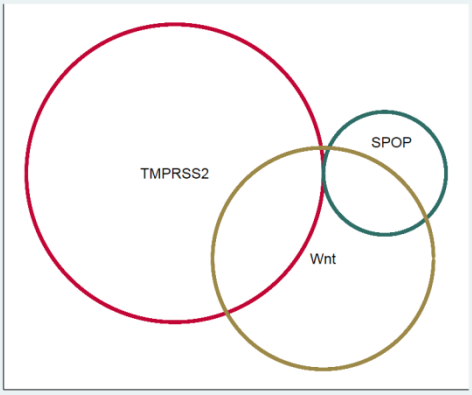
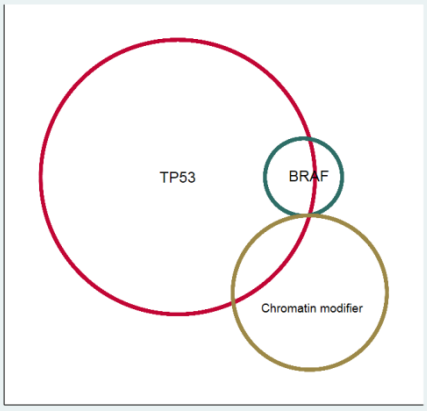
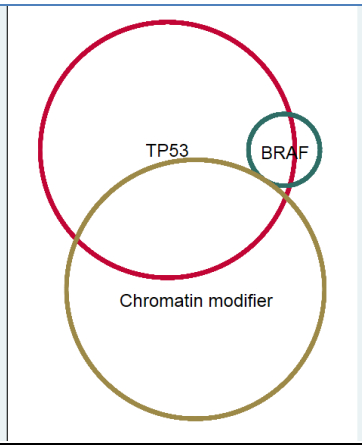
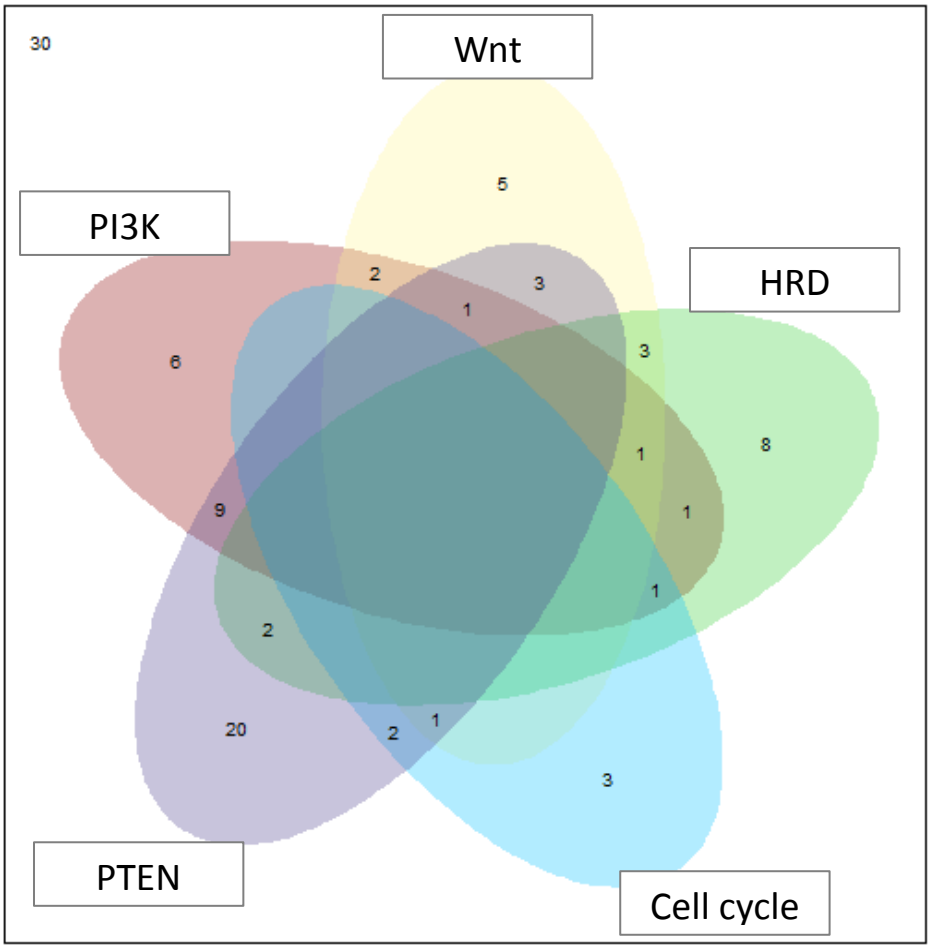

	

Figure 34e	**Figure 34f**
	

Overlap between other pathway aberrations: *PTEN* loss and known significant PI3K pathway mutations co-exist in 10 (9%) (**Figure 34a**). *TMPRSS2* rearrangements are mutually exclusive with *SPOP* mutations (**Figure 34c**). *TP53* mutations co-exist with *BRAF* mutations and aberrations in chromatin modifiers e.g. *MLL2* and MLL3 (**Figure 34e**).

Figure 37: Overlap between therapeutically relevant pathway aberrations



Overlap between therapeutically relevant pathway aberrations: 67 cancers harboured potentially therapeutically relevant aberrations and in 42 cases more than one was detected. The numbers indicate the number of samples with each pathway/gene aberration(s).

The observed degree of overlap means a hierarchical system would be required to determine allocation within a trial platform aiming to evaluate multiple molecularly-directed treatment strategies. It also limits accrual to each separate group in platforms aiming to co-recruit to multiple molecularly directed treatment strategies.

4.4 Discussion

This study assessed the feasibility of evaluating molecularly-directed treatments in men presenting with metastatic castrate-sensitive prostate cancer guided by sequencing routinely available prostate core biopsies, stored as FFPE tumour blocks. Overall, 67% of samples were successfully sequenced (192/287); 65% if the 7 samples processed and reported at lower confidence in analysis two are included (although these would be insufficient to determine trial eligibility). This test performance is comparable with the MSK-IMPACT prostate cohort, to date the largest reported series using FFPE samples in which a 68% success rate was observed (504/746)[40]. Although this cohort also included some prostatectomy samples which would be expected to have a higher success rate, as suggested by the 80% success rate reported by TCGA[30]. Similar small biopsies are sequenced in metastatic lung cancer. The Matrix trial is evaluating biomarker-treatment pairings in this setting and in 2016 reported that 70% of biopsy samples submitted are suitable for tNGS performed as part of the Stratified Medicine 2 programme (SMP2)[291]. Although consistent with the published experience of others, when considering large-scale implementation within a clinical trial the impact of a 33% test failure rate is considerable; inflating the number needed to screen, prolonging accrual time, trial duration and cost. Where biomarker prevalence is low this may render a trial not feasible. Screen-failures may also represent a potential source of bias if those successfully sequenced are systematically different to those in whom the test fails. Although limited by the relatively small sample size, our data does not suggest this was true in this study.

The process of formalin-fixation can lead to cross linking of DNA, nucleic acid fragmentation and denaturing of proteins and DNA modification, reducing nucleic acid yield and increasing the risk of sequencing artefacts[292]. For this reason, the majority of initial attempts to profile PCa cohorts mandated fresh frozen samples, considered the gold-standard for genomic research[34-36,39]. However, for molecular characterisation to be cost-efficient and clinically applicable outside of academic healthcare settings it is important to assess if routinely available FFPE tissue can be used. We therefore sought to explore potential factors influencing test performance. When comparing the results obtained by the two providers analysis two (t170) had a decreased failure rate at library construction, suggesting the additional macrodissection unique to this extraction protocol may be a useful optimisation step to increase tumour purity. However an unexpectedly high degree of inter-site

variability was observed, suggesting that pre-analytical variables were impacting on sample suitability.

A survey of pathology practices confirmed variable fixation times and longer fixation times was observed in lower performing sites. Four sites exceeded the maximum time as recommended by FM (72 hours), whilst only two sites adhered to the optimum (12-24 hours) as recommended by Genomic England (GE) [293]. Over fixation is associated with more extensive cross linking and as fixation penetration is dependent on tissue volume, shorter biopsy specific cycles typically between 3-6 hours are preferred[294]. However, as tissue processing occurs several weeks or months before trial entry is considered, it is beyond the scope of clinical trial protocols such as STAMPEDE to impact on standard practice. Within the UK, GE have recognised that the current lack of standardisation of FFPE processing is a barrier to optimising NGS protocols and are leading several initiatives aiming to improve the suitability of samples for molecular testing. Several pilots are ongoing aiming to validate different optimised tumour-specific sample handling techniques[295]. Our data supports the need for this and would suggest that if successful, this could have a considerable impact on overall sequencing success rates; out of the 18 sites submitting ≥5 samples the success rate varied hugely (0-100%) with 7 sites falling below average (67%).

Sample age was also explored as a potential factor in sequencing success. Previous reports have been contradictory and limited by small sample size, however in a pilot conducted as part of the 100,000 Genomes project, through selecting FFPE samples <6 months old and using an optimised DNA extraction protocol it was possible to perform WGS in 80% of cases, a significant improvement on the ~30% success rates previously reported[296-299]. Our results show no clear linear relationship between sample age and test performance, however considerable site variability and the performance difference between providers likely confound this assessment. Firstly, the lower performing sites contributed a larger proportion of older samples, meaning that it was not possible to disentangle the two. Secondly, younger samples were selected for analysis two which had a slightly improved success rate. Interestingly, the preliminary results from the biomarker screening pilot in which samples are between 1-4 months old (maximum permitted 8 months) are encouraging, with a success rate of over 90% (as of May-2018 40/44 samples).

The second research question addressed was to determine the prevalence of HRD in mCSPC. We hypothesised that this would be greater than observed in M0 prostatectomy cohorts but not as frequent as in heavily pre-treated or fatal mCRPC. One of the challenges

in comparing HRD prevalence estimates is that different genes may be included in the definition. In an attempt to overcome this, **Table 61** summarises relevant published data for the 14 HRD genes of interest, compiled using cBioPortal (http://www.cbioportal.org) an online resource hosted by Memorial Sloan Kettering Cancer Centre (MSK)[300-302]. Using the 14-gene HRD definition, the published prevalence estimates obtained in predominantly M0 populations are 4-24%[30,34,38,211]; whilst in advanced or fatal mCRPC it is 31-48%[31,35,36,39]. If the narrower biomarker-selection strategy is adopted (requiring pathogenic mutations in *BRCA1*, *BRCA2* or *ATM*), the published estimates are 4-12% in M0 disease and 12-21% in mCRPC. The combined results from analysis one and two are between these estimates: the HRD prevalence was 15%, *BRCA1*, *BRCA2* and *ATM* 10%; consistent with the prior hypothesis.

Of the published sequencing studies, the most relevant is MSK-IMPACT, as this contains the highest number of mCSPC cases. Although this demographic (New York) would be expected to have a higher population of men of Jewish ancestry and this likely explains the higher prevalence of *BRCA2* mutations (9% vs. 2%)[40]. It is notable that the highest HRD frequencies are observed in the rapid autopsy studies reported by Grasso *et al.* and Kumar *et al* [35,36]. Both studies achieved multi-regional metastatic sampling, sequencing 61 samples obtained from 59 patients, and 141 samples obtained from 56 patients respectively. The frequency of HRD has been shown to be highest in metastatic samples, consistent with pro-metastatic clones seeding and out-competing other sub-clonal mutations at metastatic sites[303,304]. This would also correlate with the higher frequency of HRD in metastatic cohorts[35,39,40]. The demonstration that prevalence estimates increase when more than one metastatic site is sampled supports the concept of branched evolution, leading to multiple sub-clonal populations and spatial heterogeneity[199]. In this study, only 5/286 samples were metastatic and no paired primary analysis was performed, so it is not possible to assess if spatial heterogeneity is present in *de novo* metastatic disease.

Our results add support to others who have suggested that both somatic and germline assessments are required to identify HRD PCa[40]. To date only 2 out of 40 patients screened as part of the ongoing pilot has a germline aberration in one of the 10 HRD genes tested; 5% (95% CI -1-11%), with g*BRCA2*-m detected in just 2.5% (95% CI -2 to 7%). This is lower than has reported in mCRPC; Pritchard *et al.* who reported 82/692 (12%) of men with mCRPC harboured a germline HRD, as defined by a 20 gene panel[210]. Whilst the PROREPAIR-B cohort study screened 419 mCRPC patients and mutations in *BRCA1*, *BRCA2* or *ATM* were

reported in 6%[209]. As germline *BRCA1*, *BRCA2* and *ATM* mutations are associated with a more aggressive disease phenotype and shorter responses to first-line ADT they can be expected to be enriched in advanced CRPC. However both studies may have been vulnerable to selection bias, especially Pritchard *et al.* which sampled a population pooled from seven clinical trials or precision medicine initiatives, favouring inclusion of younger patients recruited at tertiary academic institutions.

Overall, the most frequently observed aberrations were *TP53* (36%), *PTEN* (34%), PI3K pathway (18%) and Wnt pathway (14%). The prevalence of *TP53* mutation is comparable with other estimates in metastatic cohorts, including in advanced mCRPC consistent with this being an early clonal event and supported by previously reported concordance in matched archival and metastatic samples[40]. Others have shown PTEN loss is enriched in metastatic disease; consistent with this, we observed a higher frequency than seen in M0 cohorts. Our estimate is closer to that observed in mCRPC (40-50%); this also suggests PTEN loss is an early somatic event[33,40]. As observed in mCRPC, the most frequent mechanism for PTEN loss is copy number loss (25/115; 25%), with base substitutions, indels or rearrangements occurring in the remaining (10/115; 9%)[39]. The frequency of PI3K pathway aberrations is also more similar with that observed in mCRPC (~20%) than that observed in M0 populations (~7%)[30]. Wnt pathway aberrations were observed less often than in mCRPC (18-23%) but the estimate is comparable with that observed in MSK-IMPACT (15%), which has contained the largest number of mCSPC sequenced to date[40].

We observed a lower prevalence of *SPOP* mutations (4%) compared with published estimates (6-15%)[34]; this somatic mutation has been shown to be mutually exclusive of ETS-fusions, however these were present in fewer (41%) than previously reported in other cohorts (~50%) therefore cannot explain this. Instead, this finding would support the positive prognostic association suggested by data which shows a declining frequency with more advanced disease; 12% in M0, 11% in mCRPC (of which half was relapsed metastatic disease post radical treatment) and 5% in mCRPC[40]. As would be expected for a treatment-naïve population, no AR aberrations were observed. Interestingly this differs with the metastatic castrate-sensitive cases sampled as part of MSK-IMPACT which likely reflects different prior treatment as half had relapsed metastatic disease post radical treatment and all AR-aberrant cases had been exposed to prior ADT, some at this disease presentation suggesting they had developed sub-clinical CRPC[40]. Mutations in the mismatch repair genes *MSH2* and *MSH6* were observed in 3% overall, this is consistent with the prevalence

estimated in metastatic castrate-sensitive disease [40]. This suggests that this is a rare somatic aberration in mCSPC. To date, the highest frequencies of MSI have been in ductal adenocarcinoma (4 out of 10 in one small sequenced cohort), and fatal mCRPC sampled at autopsy where estimates are as high as 12%[227,228].

A strength of this analysis is that it represents the largest sequenced cohort of *de novo* mCSPC to date and reflects a population of men joining a pragmatic trial with broad inclusion criteria, increasing the generalisability of these findings. In contrast to stringent sample selection employed in other sequencing studies, we aimed to limit triaging and collect representative samples, adding to the validity of the feasibility assessment. Through the use of two different sequencing providers, these data illustrate the impact different bioinformative pipelines can have on data interpretation. When considering implementation within a clinical trial, knowledge of this potential source of variation is informative especially if a provider needs to change during the course of recruitment. Appreciating the technical challenges of tNGS and the impact of pre-analytical variables is a critical part of the feasibility assessment which was strengthened by the comparison between providers. This also enabled a greater understanding of how assays are optimised to detect different types of genetic variation (e.g. copy number alteration vs. splice variants). However in doing so, emphasises that the aim of a centralised molecular assessment suitable to guide all potential molecular-directed therapies remains ambitious.

Several limitations also need to be acknowledged, lack of paired normal tissue means that this characterisation is limited to somatic changes, with only limited germline data known from the pilot which continues. The tumour biology of PCa is recognised as a potential barrier to the implementation of molecularly-selected therapies, particularly due to spatial heterogeneity. However, through single site sampling this study cannot explore this important issue. Finally, despite including more *de novo* metastatic patients than any other published cohort, the small sample size limits the precision of the prevalence estimates; these results need to be replicated in larger cohorts.

Table 61: Contextualising prevalence data with published sequenced cohorts

	Barbieri et al	Taylor *et al.*2010	Baca *et al.*2014	TCGA 2015	Analysis One (F1)	Analysis Two (t170)	MSK-IMPACT	SU2C/PCF	Grasso *et al.*2012	Kumar *et al.*2016
	M0	**Mostly M0**	**Mostly M0**	**M0**			**Mixed**	**mCRPC**	**Fatal mCRPC**	**Fatal mCRPC**
	n=112	**n=103**	**n=57**	**n=333**	**n=115**	**n=77**	**n=451**	**n=150**	**n=59**	**n=56**
ATM	1.8%	0%	7%	5%	6%	10%	2.2%	5%	8%	9%
BARD1	0%	1%	0%	0.3%	0%	0%	0.4%	2.7%	1.7%	5%
BRCA1	1.8%	1%	0%	0.6%	0%	0%	0.9%	0%	0%	5%
BRCA2	0%	5%	7%	1.5%	2%	3%	6%	8%	10%	11%
BRIP1	0%	1.9%	0%	1.8%	0%	0%	0.9%	2%	5%	5%
CDK12	0%	0%	0%	1.5%	6%	4%	6%	4%	5%	7%
CHEK2	0%	0%	1.8%	3%	0%	1%	0.4%	2%	1.7%	5%
NBN	0.9%	5%	7%	6%	0%	0%	5%	10%	29%	21%
PALB2	0%	0%	0%	1.2%	0%	0%	0.4%	0.7%	1.7%	9%
Rad51	0%	0%	4%	2.1%	0%	0%	0.7%	2%	1.7%	4%
Rad51B	0%	1.9%	0%	1.5%	0%	0%	0.2%	0.7%	0%	1.8%
Rad51C	0%	1.9%	0%	0.3%	0%	0%	0.4%	0.7%	1.7%	9%
Rad51D	0%	1%	0%	1.5%	0%	0%	0	0%	0%	4%
Rad54L	0%	0%	0%	0.6%	0%	0%	0.2%	0%	0%	7%
Samples sequenced (n)	112	103	56	333	115	77	501	150	61	141
HRDm (n)	5/112	12/103	12/56	81/333	16/115	12/77	85/448	47/150	27/59	27/56
HRD (95% CI) (%)	**4 (1-7%)**	**12 (7-17%)**	**21 (12-30%)**	**24 (20-28%)**	**14 (9-19%)**	**16 (9-23%)**	**19 (16-22%)**	**31 (25-37%)**	**46 (35-57%)**	**48 (37-59%)**
BRCA or ATM (n)	4/112	6/103	7/57	22/333	9/115	9/77	42/451	18/150	10/59	12/56
BRCA or ATM (95% CI (%))	**4(1-7%)**	**6(2-10%)**	**12 (8-16%)**	**7 (5-9%)**	**8(4-12%)**	**9(4-13%)**	**9% (7-11%)**	**12(8-16%)**	**17(9-25%)**	**21(12-30%)**

Summarised from www.cbioportal.org [301,302]

Table 62: Contextualising prevalence data

		Published data MSK-IMPACT n=140	Analysis One (F1) n=115	Analysis Two (t170) n=77
Pathway	***Gene***			
	AR	4%	0%	-
	PTEN	18%	34%	-
	TP53	30%	36%	-
	RB1	7%	1%	-
DNA repair	BRCA2	7%	2%	3%
	BRCA1	1%	0%	0%
	ATM	2%	5%	10%
	FANCA	3%	0%	0%
	CDK12	6%	5%	4%
	MSH2	2%	2%	-
	MLH1	1%		-
PI3K pathway	PIK3CA	4%	7%	-
	PIK3R1	4%	3%	-
	*AKT*1	1%	1%	-
	*AKT*3	0%	1%	-
Wnt pathway	*APC*	14%	7%	-
	CTNNB1	6%	7%	-
	RNF43	1%		-
MAPK pathway	BRAF	4%	3%	-
	HRAS	1%		-
	KRAS	1%	1%	-
Chromatin remodelling	KMT2A	1%		-
	KMT2C	9%	3%	-
	KMT2D	4%		-
	KDM6A	2%		-
Others	IDH1	0%	1%	-
	SPOP	11%	4%	-
	FOXA1	10%		-

4.5 Conclusion

Through profiling a sample of men enrolled in the STAMPEDE trial, this study demonstrates that it is feasible to use prostate core biopsies stored as FFPE to perform tNGS. However, whilst the test failure-rate is comparable with other published examples, it still poises challenges to evaluating treatments in low prevalence biomarker-defined groups. HRD is present in around 15% (95% CI 11-19%) of metastatic castrate-sensitive tumours which is less frequent than observed in mCRPC, but more common than reported in M0 disease. When considering the evaluation of PARPi in this setting, the evidence of predictive effect needs to be balanced against the prevalence of the biomarker. Currently, *BRCA2* mutations have the strongest evidence of predictive effect; however our data has shown these are rare in the STAMPEDE population (2%). The trial design will be informed by emerging data as to the strength of the predictive effect of HRD, which will in turn influence the appropriate target effect size. Our data highlight the many challenges of evaluating multiple biomarker-selected questions within one MAMS platform. The mutational prevalence remains low and may overlap, reducing the number of eligible patients. Our clinical knowledge remains incomplete and the majority of variants are of unknown significance. It is hoped that as more treatments are shown to benefit biomarker-selected groups, the continued rapid expansion in genomics technology and research will enable trials such as STAMPEDE to adapt to evaluate this promising strategy.

Chapter 5 Discussion and Future directions of research

Advances in the understanding of the molecular basis of metastatic prostate cancer may explain the clinical heterogeneity observed and provide a biological rationale to move away from a uniform approach to management. The results of the STAMPEDE trial have shown three additional systemic therapies improve OS when compared with ADT alone; docetaxel, celecoxib-ZA and abiraterone. All differ in therapeutic mechanism of action, toxicity and cost, such that variable individual patient benefit is anticipated from each approach. In this thesis I sought to develop a further understanding of the results of the STAMPEDE trial. I aimed to explore how trial data may inform treatment selection and consider how the trial may adapt to evaluate biomarker-directed treatment strategies in the future.

5.1.1 Contextualising the STAMPEDE celecoxib - zoledronic acid results

I undertook a systematic review and meta-analysis aiming to contextualise the intriguing and least well-understood finding of the trial so far, the beneficial effect of celecoxib-ZA in men with metastatic castrate-sensitive prostate cancer. The main challenge faced when trying to understand this result is the lack of supporting clinical data. Despite extending searches to include non-randomised data no studies evaluating the combination of a cox-2 inhibitor and a bisphosphonate were identified; whilst uncertainty surrounding bisphosphonate use in those studies found means the relevance of the available data may be limited. This further emphasises that further research is needed if the findings of this comparison are to impact practice. Secondly, my findings illustrate the impact the cardiovascular toxicity data observed in two adenoma prevention trials had on the evaluation of cox-2 inhibitors. The demonstration that celecoxib-ZA acid could be a life-prolonging, palliative therapy calls into question whether the response to these data was proportionate and highlights the need to contextualise efficacy and safety data. Finally, the value of adopting a meta-analysis approach is again shown in the recent publication that confirms that, contrary to concerns at the time, cardiovascular toxicity is limited to rofecoxib and is not a class effect. Taken together, my results add support to the need to further evaluate the combination of celecoxib-ZA.

In the absence of supporting clinical data, the case for future trials is strengthened by work showing a potential mechanism for the observed additive, therapeutic effect. It has been shown that both celecoxib and ZA have an immunomodulatory role and I suggest a hypothesis whereby both treatments act to promote an anti-cancer effect mediated by $\gamma\delta$ T

cells. Unfortunately, this hypothesis cannot be explored retrospectively in the samples available (which are limited to FFPE prostate core biopsies). This highlights a challenge of expectant collection of samples which may often be inappropriate for the hypotheses and technologies available at the time of planned analysis. Instead a new trial would be required with prospective sampling, to include collection of peripheral blood mononuclear cells (PBMCs) to permit analysis of γδ T cells. Whilst exploratory, the hypothesis proposed describes a mechanism that is not specific to PCa therefore evaluation in other solid organ cancers with metastatic bone involvement may be considered. Of note, γδ T cells have been shown to exert an anti-cancer effect in lung and breast cancer, providing a rationale to consider expanding to these indications if the results of STAMPEDE are confirmed[305,306]. Several ongoing trials of celecoxib with immunomodulatory agents including checkpoint inhibitors, vaccines and rintatolimod, a restricted Toll-Like Receptor 3 (TLR3) agonist, may also be informative. Many of these trials include translational research that may help understand the immunomodulatory role of celecoxib and help define the population in which celecoxib-ZA may be of benefit.

Informing approaches to future research

Adaptive trial designs, such as that used in the STAMPEDE study, are ideal to evaluate therapeutic combinations and address pre-specified biomarker-focused questions. Multiple arms enable agents to be assessed alone and in combination, whilst the use of a shared control arm permits analyses of proposed predictors of differential response. The overlapping putative oncogenic drivers demonstrated in the sequencing analyses conducted as part of this work provide a strong rationale to evaluate combination therapies in this genetically heterogeneous disease. However, addition treatments can be expected to come with increased cost and toxicity and therefore are likely to only be indicated in a subset where the risk-benefit and health economic analysis is favourable. For example, analyses within the abiraterone comparison may identify a biological subset with a differential treatment response which could then be validated within the enzalutamide and abiraterone comparison, providing justification for this intensified treatment option.

Well-designed translational sub-studies conducted in parallel with clinical trials can make the most of the clinical data collected as part of the evaluation of the primary outcome, whilst also generating and testing hypotheses to explain differential treatment effects. Identifying biomarkers predictive of differential treatment effects within the abiraterone and docetaxel treated groups is an ongoing research aim of the STRATOSPHERE

(STratification for RAtional Treatment-Oncomarker pairings of STAMPEDE Patients starting long –term Hormone treatment) consortium. Supported by preliminary data provided by me and others, this consortium has been awarded a programme grant from Prostate Cancer UK. Having initiated sample retrieval from STAMPEDE sites, I continued to work with the trial team in establishing a trial biorepository to support future biomarker studies. Focusing on the docetaxel and abiraterone comparisons, we have obtained samples from over 1200 patients to date. The STRATOSPHERE protocol outlines the proposed analyses focusing on the docetaxel and abiraterone FFPE blocks, germline DNA extracted from saliva samples, as well as exploration of resistance mechanisms through sequential ctDNA collection. It is hoped that through future adaption prospective validation and evaluation of potential therapeutic pairings can be achieved within the STAMPEDE trial platform, for example through biomarker-stratified randomisations and testing of biomarker-directed therapies through the addition of new research arms.

5.1.2 PSA kinetics as prognostic biomarkers

The feasibility and prevalence sequencing study highlights some of the complexities in developing and implementing genomic biomarkers, which continue to be costly, logistically challenging and reliant on specialist expertise. In comparison, PSA-based biomarkers have many advantages; they are readily available, inexpensive and measured using fully analytically validated assays, routinely provided in local laboratories. Pragmatically, PSA levels are understood by clinicians and men with PCa meaning that PSA based outcomes could be relatively easily incorporated into clinical practice and at little extra cost. I sought to evaluate two PSA outcomes as additional prognostic biomarkers at two clinically meaningful time points such that they may be able to inform the use of different treatment strategies in the castrate-sensitive setting[307].

The magnitude of PSA response evaluated in the first 12 weeks of ADT treatment and PSA nadir values assessed shortly after completion docetaxel were both shown to associate with survival differences. Using established threshold values, the strongest evidence of prognostic effect was seen for PSA nadir which remained statistically and clinically significant in a multivariable analysis. PSA was also shown to associate with OS, although statistical significance is reduced when accounting for absolute PSA, suggesting that future prognostic tools should consider evaluating this as well. Consistent with the prior hypothesis, the value of PSA based outcomes appears greatest in castrate-sensitive disease, compared with castrate-resistant setting.

A PSA nadir of >4ng/mL in patients receiving ADT + docetaxel is shown to provide additional prognostic information at a clinically relevant time point shortly after completion of docetaxel. Several ongoing trials are evaluating ADT + docetaxel + AR-targeted agents and should this intensified approach be shown to be effective, risk-stratification post docetaxel will be important in identifying high-risk groups in whom the additional toxicity and cost can be justified. Prospective validation of this prognostic biomarker will be important; this could be achieved through randomising men shortly after completion of SOC docetaxel comparing ADT vs. ADT + AR therapy stratified by PSA nadir. This would permit pre-planned subgroup analyses within these proposed risk categories. It may be possible to explore this within a further retrospective analysis using STAMPEDE data as a proportion of patients allocated to the enzalutamide and abiraterone comparison from Dec-2015 onwards will have received SOC docetaxel, permitted by way of protocol amendment. The protocol guidance reflected the timelines for the docetaxel-randomised cohort and recommended docetaxel was commenced within around 12 weeks after starting ADT. In patients allocated to receive abiraterone and enzalutamide, treatment commenced after docetaxel had been completed. The PSA nadir value was calculated in the same way and therefore likely reflects the PSA response to ADT + docetaxel. Therefore in 2020 when the results of the primary efficacy analysis are reported, it may be possible to stratify by PSA nadir category and explore this effect further.

The results of the PSA response analysis are more exploratory. Future work is required to validate the optimum threshold value that offers the best discrimination. A larger sample size may permit analyses that include both PSA response and absolute PSA. The magnitude of PSA response appears prognostic but to influence the clinical decision to give docetaxel it is important to determine if patients experiencing a PSA response of ≥99% on ADT alone derive the same benefit from docetaxel. The STAMPEDE trial offered a unique opportunity to analyse PSA response within a cohort randomised to receive ADT or ADT + docetaxel, but unfortunately the data collected as part of the current protocol does not permit this. Moving forward, PSA measured close to the time of randomisation could be collected however it would be challenging to explore treatment interaction as docetaxel has become SOC and use is no longer randomised. Adjustment would be needed for differences in age, disease burden, baseline performance status and co-morbidity that currently impact on the decision to use docetaxel. Additionally, statistical power to explore this in the STAMPEDE cohort would be limited by the small proportion (~10%) within the randomised population who are now not planned to receive docetaxel. Retrospective collection of PSA at

randomisation from the previously randomised docetaxel cohort within STAMPEDE would permit this analysis, and the resource required for this may be considered justified by the significant prognostic impact observed in patients receiving ADT alone.

PSA transcription is AR-dependent and as such it is a measure of a biological process. This provided the rationale for the hypothesis that rapid PSA decline could be used as a clinical surrogate for a biological subset of exquisitely hormone-sensitive disease. My analyses demonstrate that men who experience early disease progression on ADT alone have a 3 year survival-rate of just 20% and median survival time is considerably reduced in comparison to those who reached the landmark (24 weeks post randomisation) progression free (17 versus 45 months). A lack of PSA response may reflect poor therapeutic response or a pre-existing biological variant. For example, early progression on ADT may represent an inherent, aggressive subtype that is inadequately treated by ADT alone. Men experiencing a poor PSA response were more likely to have presented with a lower PSA which has been associated with a ductal non-PSA secreting subtype[308]. Sequencing studies have shown a higher frequency of MSI associated with ductal carcinoma[227]. Analyses of pre-treatment FFPE blocks using tNGS assays that include assessments of MMR genes and mutational burden would enable this hypothesis to be tested. A high PSA nadir was also shown to be associated with an increased risk of death: a PSA nadir of >4ng/ml despite docetaxel is associated with a 2-fold increase risk of death (HR 2.64; 95% CI 1.64-4.26, $p<0.0001$) compared with a PSA nadir ≤0.2ng/ml. Correlative analysis of this group would enable hypotheses to be developed that may explain docetaxel resistance and/or differential benefit from abiraterone which is likely to only be cost-effective in those deriving least benefit from docetaxel, the current SOC. Developing predictive biomarkers to characterise these group at diagnosis would help guide the use of additional treatments aiming to identify the optimum treatment strategy for these groups shown by my work to be at high-risk of poor survival outcomes.

The clinical utility of PSA nadir and the optimum threshold value requires prospective validation. Threshold values are often arbitrarily defined and inadequately interrogated, which risks undermining biomarker specificity. However understanding the relationship between a continuous biomarker and outcome requires a large trial sufficiently powered to assess the interaction, typically estimated to be four times larger than a randomised treatment evaluation. The RxPONDER trial (Rx for Positive Node, Endocrine-Responsive Breast Cancer; NCT01272037) is an impressive example of this. This NIH sponsored trial will

address whether recurrence score (RS), as reported by a gene expression assay using a continuous scale 0-100 (Oncotype Dx, Genomic Health, Redwood City) can be used to identify breast cancer patients who can safely avoid adjuvant chemotherapy. Patients are eligible if RS score is <25 and randomisation is between endocrine therapy, with or without chemotherapy. If a significant interaction between RS score and chemotherapy benefit is shown, the trial will determine the optimum threshold value for recommending chemotherapy using a pre-specified statistical algorithm. Importantly, an additional comparative effectiveness analysis will evaluate the benefit of testing e.g. whether the resource saving of chemotherapy avoidance justifies the cost of testing. 10,000 women have been randomised, costing the NIH $28 billion, in addition to significant insurance based funding to support testing, at up to $4000 per test[309]. The methodology employed by RxPONDER has itself has been evaluated in a separate comparative effective analysis. By virtue of being able to evaluate the use of a costly test and potential treatment avoidance, together with collecting survival, DFS and patient reported outcomes, the expected value of the research presents a return of 17-39 times for the public funders of the study[309].

PSA nadir >4ng/ml is proposed as an inclusion criterion for future trials evaluating intensified maintenance therapy post-docetaxel in the castrate-sensitive setting. A similar strategy has already been explored in a small non-randomised phase II study, albeit not using the updated treatment paradigm. The S1014 trial evaluated abiraterone in patients experiencing a suboptimal PSA response to ADT (similarly defined as >4ng/ml) assessed 6-12 months after initiation[193]. My work would support future trials prospectively evaluating PSA nadir as an on treatment prognostic biomarker through randomising to ADT alone or intensified treatment e.g. addition of AR-targeted agent following completion of docetaxel. This could be assessed within STAMPEDE and may be possible alongside future comparisons of treatments that cannot be safety given concurrently with docetaxel. The timing of randomisation would move to shortly after completion of docetaxel, as would be proposed for any future PARPi evaluation. An enrichment design, limiting recruitment to PSA>4ng/ml would be preferable. As shown by our results, this group has the shortest time to CRPC progression and death so information could be acquired in the shortest time. Results from this work enable this to be modelled and would inform this design. This prospective validation could help the translation in clinical practice of the findings of the abiraterone comparison, and potentially, if shown to be beneficial, the enzalutamide and abiraterone combination.

5.1.3 Genomic biomarkers: feasibility and prevalence assessment

Precision medicine initiatives reliant on characterising molecular predictive biomarkers are proving challenging to implement, due in part to incomplete knowledge. My aim was to acquire preliminary data to inform the best approach to evaluating treatments such as PARPi, hypothesised to benefit a genetic subset of mCSPC.

The findings of this study show that, whilst routinely available FFPE prostate core biopsy samples may be used for tNGS, considerable variability in sample quality is observed and test failure rates continue to be a barrier to the cost-efficient evaluation of low-prevalence biomarker groups. These data emphasise the importance of obtaining feasibility data in clinically representative samples. The variability between sequencing providers also illustrates the complexities of sequencing data processing and the impact this can have on the biomarker specificity. It is a source of frustration that crucial details of the bioinformatic pipeline may not be sufficient well described when clinicians and trialists come to use these results to inform clinical decision making. My work highlights the impact different technical protocols, analytical processes and data interpretation can have. Indeed, this likely explains why regulatory approval of many biomarker-selected treatments is accompanied by a specified biomarker and provider. This poses several challenges to trial conduct. The field of genomics is in flux; assays, technologies and the knowledge base of germline variants (i.e. normal), and somatic variants of potential clinical significance, are all undergoing constant refinement. Trial protocols are required to define if the biomarker measurement is to be fixed or may evolve; the former defines the population as would be required for regulatory submission, but may limit the relevance of the results if assay thresholds and sensitivity change. Furthermore, costly equivalence studies are likely required should the biomarker provider change during the conduct of a trial.

Consistent with the prior hypothesis, results demonstrate that the prevalence of HRD is less than observed in mCRPC but more frequent than seen in indolent, localised disease. Given the challenges of genetic characterisation, I sought to clinically characterise the HRD subgroup. Identifying clinical features may be able to supplement genetic data and, much like platinum-sensitivity in the context of ovarian cancer could be a useful adjunct in identifying patients with this disease subtype. However, unfortunately numbers remain small and findings are preliminary at this stage. Interestingly, in contrast to the prior hypothesis, the HRD subgroup were no younger in age at presentation of metastatic prostate cancer. This would suggest that the majority of HRD mutations are acquired as

somatic mutations as opposed to an inherited predisposition, consistent with the low frequency of germline HRD aberrations detected in the ongoing biomarker screening pilot.

Evidence of prognostic effect can inform the design of biomarker-enriched trials and impact on the assessment of feasibility, particularly of low-frequency biomarkers. When considering biomarker-selected randomisation within a platform trial, this also informs whether it is justifiable to share a control arm, with resulting efficiencies. If the biomarker is known to be prognostic, separate comparisons are required in order to distinguish predictive from prognostic effect, as exemplified by the design of FOCUS-4[310]. Knowledge of prognostic effect can also inform the size of a trial powered to detect in a difference in time-to-event outcomes. If the survival time is shorter, the information required for reliable analyses (e.g. number of deaths for a trial powered on primary outcome of survival) will be accrue sooner and therefore a smaller trial may be required. It is a limitation of this study that prognostic impact could not be explored as treatment varied and follow-up was insufficient. This is an aim of ongoing translational work being conducted as part of STAMPEDE and the association STRATOSPHERE consortium.

Prevalence data was recognised to be of crucial importance in understanding the scope of benefit biomarker-driven therapies could have, and the optimum way in which to evaluate these in a trial. We observed the 'long tail' of somatic mutations that mostly occur in <10% of patients. This represents a challenge in the design of randomised biomarker-driven trials and means design approaches originally recommended for rare diseases may become relevant. In a recent published framework, Parmar *et al*. recommend considering extending collaborations to broaden recruitment potential and ensuring the experimental and control arms are as different as possible. They also suggest considering a more information-heavy outcome measure, or finally, reviewing target effect, power and the alpha (type I error rate)[311]. Unique to this setting of biomarker-selected trials is the costs incurred with the high screening burden. However, this is not a challenge specific to prostate cancer; in a precision initiative led by MD Anderson 6.4% of those screened were paired with a targeted drug[312]. As of July 2017, 5,963 patients have been screened as part of the NCI-MATCH trial, but only 18% of patients harboured a mutation and overall the prevalence has been lower than anticipated, resulting in only 8 out of 30 treatment arms reaching the minimum patient accrual of 35[313]. Indeed, the denominator of patients undergoing molecular analysis is often not captured in precision medicine reports, undermining the assessment of feasibility and cost-effectiveness of this approach [314].

HRD prostate cancers remain the only genetically-defined subset for which a paired treatment strategy has been shown to be beneficial[57]. There are emerging data to support evaluating other biomarker-treatment pairings, but these remain at an early stage and predictive biomarkers may be relatively less well defined. For example, the Canadian Cancer Trials Group has established the Prostate Cancer Biomarker Enrichment and Treatment Selection (NCT03385655) study as a master protocol that facilitates screening using cell free DNA, matching participants to a series of spin-off non-randomised phase II trials. Only one trial is registered to date, evaluating palbociclib, a CDK4/6 inhibitor in mCRPC with *CCND1* gain/amplification or *RB1* loss (NCT02905318). More often treatment and predictive biomarkers are at different development stages, meaning instead trials enrol unselected populations with pre-planned biomarker characterisation. Trametinib, a MEK 1/2 inhibitor, is under evaluation in unselected mCRPC (NCT02881242) due to the lack of a predictive biomarker. Instead, mandated pre-treatment and progression metastatic sampling, together with ctDNA analysis, will aim to identify molecular correlates of treatment sensitivity and resistance. At the time of writing, the only other biomarker-treatment pairing undergoing randomised evaluation is an AKT-inhibitor (AZD5363); the RE-AKT trial will randomise 100 men stratified by PTEN loss to receive enzalutamide with AZD5463 or placebo (NCT02525068). This follows the demonstration that Ipatasertib (AKT-inhibitor) and abiraterone prolongs radiological PFS (rPFS) in mCRPC, with the greatest treatment effect observed in cases with PTEN loss as determined by IHC; although benefit was also seen in those without this proposed predictive biomarker. Therefore, whilst there is much enthusiasm for biomarker-directed therapy in prostate cancer, the strength of clinical data to support this strategy is limited at this time.

Well-designed clinical trials are needed to evaluate the numerous putative predictive biomarkers and provide evidence of the strength of the predictive effect. In their review, Freidlin and Korn propose that clinical trial design should reflect the prior strength of evidence[315]. Very strong evidence that benefit will be limited to the biomarker positive subset is required to support an enrichment strategy where enrolment is limited to biomarker positive patients. Efficiencies are to be gained, as large treatment effects are anticipated, requiring a smaller sample size; although where the biomarker prevalence is low the screening burden will remain high. Where there is less certainty, biomarker-stratified trial designs may be employed, evaluating both biomarker positive and biomarker negative groups in parallel or sequentially. Caution is needed in subgroup interpretation and protecting against false positive results (Type I error) with this approach. Parallel

assessment of biomarker defined subgroups may be undertaken but this reduces the power to detect an effect group as a whole, in the case the biomarker is not predictive. Low biomarker prevalence may also compromise the power in the biomarker positive subgroup. Sequential approaches, such as testing efficacy in the biomarker positive subgroup and then in the whole group may be used, however this risks inappropriately recommending treatment in the broader group, when in fact the benefit is limited by the subset. The Marker sequential test design (MaST) is proposed to mitigate this risk. Treatment efficacy is assessed first in the biomarker positive group at a reduced significance level, then, if beneficial, assessed in biomarker negative subgroup. Efficacy is only assessed in the whole group if no benefit has been seen in the biomarker positive group[316].

As discussed above, putative predictive biomarkers and paired therapies often develop at a different pace. Biomarkers of uncertain predictive effect may be tested using a 'fall-back' design. Recruitment is unselected and the trial is powered to detect a difference in the whole group. Pre-planned subgroup analysis of the biomarker positive subset is performed, but only if results are negative in the whole population. This is a useful strategy to prevent a conclusion being drawn that a treatment is ineffective, when in fact it is beneficial in a subset. Pre-specifying this subset is important to avoid the criticisms and type 1 error rate of post-hoc analyses. Low biomarker-prevalence will continue to be a challenge however, as the power in the biomarker positive subset will remain lower than in the biomarker negative group for the majority of biomarkers, leading to longer trials. As such, ongoing efforts to provide prevalence data relevant to this disease setting are valuable, enabling these scenarios to be modelled and appropriately selected. Two challenges remain with the approach suggested by Freidlin *et al.* Firstly, how should the strength of the biomarker be determined? Secondly, given the time and cost of clinical trial design and set-up, how can trialists keep up with the pace at which biomarkers are being defined, re-defined and evidenced? One approach is to adopt a pragmatic approach: would randomisation of an unselected population be acceptable and therefore feasible? In answer to the second question, adaptive multi-stage protocols that incorporate lack-of-benefit interim analyses to determine ongoing accrual, including biomarker subgroup specific rules, are likely to be the most efficient.

5.1.4 Future work

The work presented in this thesis highlights many of the challenges relevant to the implementation of stratified medicine but also demonstrates the strengths and opportunity of conducting translational studies in parallel with clinical trials.

As the celecoxib-ZA result demonstrates a statistically and clinically significant benefit observed within a pre-planned sub-group analysis it should be considered relevant and robust. However the impact of this finding is undermined by the lack of a clear mechanistic rationale for the combination. My work is speculative as there are no biological samples in which to test hypotheses and in retrospect this was a real missed opportunity within a study that evaluated celecoxib, ZA and the combination with shared controls. This has implications for future evaluate combination therapy which should adopt a MAMS design and conduct parallel translational programmes to collect supportive data test hypothesises and improve understanding of mechanistic interactions. This in turn can be used to rationally identify biologically defined sub-groups with differential treatment response, where the toxicities and cost of combination therapy may be justified.

My work has shown PSA-based outcomes to be pragmatic prognostic biomarkers able to improve risk stratification. Adaptive trials such as STAMPEDE can characterise biomarker defined sub-sets and through subsequent randomisations, seek to prospectively validate these findings within the same overarching protocol. Identifying high risk sub-groups may in turn enable new treatment strategies to be evaluated in smaller, quicker trials where signals can be sought in groups shown to have the highest unmet clinical need. For example, post docetaxel maintenance therapies may be first evaluated in patients with a high PSA nadir. Should a FFS benefit be observed at an interim analysis, randomisation broadened to include all patients supported by the initial indication of activity.

Review of the current published data shows that there is currently insufficient evidence to support prospective molecularly selected therapeutic selection in mCSPC. My work has highlighted many of the challenges, including a relatively high failure rate and the importance of assay validation and comparability. Individual putative drivers occur in low frequencies but often co-exist. Knowledge as to which are the key oncogenic drivers remains incomplete and it is likely that many of those identified are passenger mutations or only relevant to sub-clonal populations. The impact of heterogeneity needs to be assessed before we can be confident to as to the reliability of directing therapy based on a single

core biopsy obtained from a patient with metastatic disease. Approaches such as ctDNA are appealing as they avoid some of these sampling issues. However this strategy is best developed in the setting of relapsed mCRPC where yield is likely to be high. It remains technically challenging to sample men with mCSPC who are typically reviewed by oncologists shortly after starting ADT when the yield of ctDNA will be decreasing rapidly. Validating ctDNA approaches in this setting would seem the next priority in advancing molecularly targeting therapy in mPCa. Then, as has typically occurred in the past, this strategy can be brought forward and evaluated earlier in the disease course if benefit is seen. This would justify the likely significant changes in the clinical pathway required e.g. ctDNA collection by urologists as soon as a histological diagnosis is made and prior to initiation of treatment.

Whilst data to support a MAMS trial that recruits to molecularly defined cohorts remains lacking, it is still possible for trials such as STAMPEDE to acquire data to support a more individualised approach to treatment. This can be achieved through parallel translational research that aims to further mechanistic understanding and generate and test hypothesis to explain differential treatment effects. MAMS trials are ideally designed to support such research as the use of shared controls allows several experiments to be conducted to explore predictors of response and toxicity. The evaluation of agents alone and in combination represents a particular opportunity e.g. analyses within the abiraterone comparison could aim to identify a sub-set that derive more benefit from the single agent and this this could be validated within the combination abiraterone + enzalutamide. Or conversely a population with a poor response could be identified and a hypothesis tested that the biology of this sub-group harboured a resistance mechanism that could be overcome by the addition of enzalutamide. Importantly, academic trials can seek to explore predictor where there is less commercial drive to do so e.g. AR-targeted therapies where response rates in unselected populations are sufficient high to de-motivate, but the health economic need remains high. Specific to STAMPEDE is the ability to test predictors of differential response to abiraterone and docetaxel, both now SOC treatments. This is possible using archival diagnostic samples from the randomised comparisons to each, including a proportion who were randomised contemporaneously to either. Accepting the limitations highlighted of using primary prostate samples, this remains a priority and highlights again the ability of un-stratified trials to acquire data to support a more stratified approach in the future. Overall the aim should remain the same: we should identify men

who are benefiting least from the current treatment strategies in order to focus and accelerate research to improve outcomes in those with the highest unmet clinical need.

5.2 Conclusions

A systematic review was unable to identify any supporting data for the observed beneficial effect of celecoxib-ZA. When seeking to understand the STAMPEDE results, an immunological hypothesis is proposed by which celecoxib-ZA may interact via promoting γδ T cells to exert an anti-cancer effect and modulate the metastatic niche. Two novel prognostic biomarkers are proposed, relevant to the updated treatment paradigms; the magnitude of PSA response on ADT alone and PSA nadir assessed following docetaxel. These assessments are made at clinically meaningful time points and results suggest that both are prognostic of overall survival in the STAMPEDE cohort. Further validation is required but if confirmed, these may have clinical utility in informing the use of docetaxel and subsequent post-docetaxel maintenance therapies. Sequencing data from 192 patients with mCSPC was successfully obtained through two industry collaborations. Consistent with the prior hypothesis, the prevalence of HRD was observed to be less than reported in mCRPC but greater than seen in M0 disease. The evaluation of biomarker-treatment pairings continues to be challenged by low biomarker prevalence, compounded by test failure rates and a lack of validated intermediate clinical endpoints, which together necessitate long clinical trials with a high screening burden. However as shown by the data presented, additional analyses within large clinical trial cohorts can provide valuable data supporting biomarker development and informing trial design in this disease setting.

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Appendices

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A1. Summary of study reports ineligible for inclusion in meta-analysis

A1: Table 63: Summary of ineligible NSCLC trials

Publication	Setting	Article type	Accrual			Trial design		Treatment	Trial status	Reason for exclusion
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration		
Zhou *et al.* 2007[140]	Metastatic 1st line	Abstract only (full article in Chinese)	China	NK	65 (NK)	Phase II	SOC chemo +cox-2 inhibitor SOC chemo	Celecoxib 400mg BD	Completed	Insufficient data
Xiong *et al.* 2007[317]	Metastatic 1st line	Abstract only (full article in Chinese)	China	NK	60 (NK)	Phase II	SOC Chemo + cox-2 inhibitor vs. SOC Chemo	Celecoxib 400mg BD	Completed	Insufficient data
Liu *et al.* 2012[318]	Metastatic 1st line	Abstract only (full article in Chinese)	China	NK	46 (NK)	Phase II	SOC Chemo + cox-2 inhibitor vs. SOC Chemo	Celecoxib 400mg BD		Insufficient data
Pei *et al.* 2014[319]	Metastatic 1st line	Abstract only	China	2010 to 2012	81 (NK)	Phase II	SOC Chemo + Cox-2 inhibitor vs. SOC Chemo + placebo	Celecoxib 400mg BD		Insufficient data Authors contacted, no response
Zhou *et al.* 2007[140]	Metastatic 1st line	Abstract	China	NK	65 (NK)	Phase II	SOC chemo +cox-2 inhibitor SOC chemo	Celecoxib 400mg BD		Insufficient data

A1: Table 64: Summary of ineligible trials in non-metastatic breast cancer

Publication	Setting	Article type	Accrual			Trial design		Treatment
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration
Chow *et al* 2005 CAAN trial [133,320]	Localised; neo-adjuvant and Adjuvant	Full	Japan & Hong Kong	2002 to 2003	41 (90)	Phase II/III	Exemestane + cox-2 (Group A) Exemestane (Group B) vs Letrozole (Group C)	Celecoxib 400mg BD for 3 months pre-surgery and at least 2 years in adjuvant setting
Ahmadloo N *et al* 2009[321]	Localised; neo-adjuvant	Abstract only	Iran	NK	50 (NK)	Phase II	SOC chemo + cox-2 SOC chemo + placebo	Celecoxib 100mg BD
Pierga *et al*[322]	Localised; neo-adjuvant	Full	France	2004 to 2007	220 (NK)	Phase II	Chemo (docetaxel) Vs. Chemo (docetaxel) + cox-2	Celecoxib 400mg BD
Rosati *et al*.2011[323]	Localised, adjuvant	Abstract only	Italy	2003 to 2006	182 (NK)	III	Anastrozole + placebo vs. Anastrozole + etorcoxib	Etorcoxib
Higgins *et al*.2012[135]	Localised, adjuvant	Abstract only	Multi-national	NK	1622 (NK)	Phase III, factorial	Exemestane vs. Anastrozole 2nd randomisation: celecoxib vs. placebo	Celecoxib 400mg BD
Brandao *et al*.2013[150]	Localised, neo-adjuvant	Full	Holland	2005 to 2007	37 (NK)	Phase II	Cox-2 vs. placebo	Celecoxib 400mg BD

A1: Table 65: Summary of ineligible trials in non-metastatic breast cancer -2

Publication	Setting	Article type	Accrual			Trial design		Treatment
			Country	Date	Total accrual	Phase	Comparison	Cox-2 dose & duration
Samuel *et al.*2014[324]	Localised, adjuvant	Full	USA	2004	327	Phase III 2x2 factorial	SOC chemo vs. non-standard chemo (AC vs. FEC-100) With and without addition of cox-2 inhibitor	Celecoxib 400mg BD
Aristarco *et al.*2016[325]	Localised, neo-adjuvant	Full	Italy	2004 to 2009	125	Phase II	Exemestane vs. celecoxib vs. Placebo	Celecoxib 400mg BD
Rea *et al.*2016 NEO-EXCEL[326]	Localised, neo-adjuvant	Abstract	UK & Canada	2016	266	Phase III factorial	Exemestane vs. Letrozole 2nd randomisation: Celecoxib vs. placebo	Celecoxib 400md BD
Giacchetti *et al.*2017[327]	Locally advanced, neo-adjuvant	Full	France	2004-2007	340 in total; 220 within strata 1	Phase III HER-2 stratified	Strata 1 HER2 negative: Chemo vs. chemo + celecoxib Strata 2 HER2 positive: Chemo vs. Chemo + trastuzumab	Celecoxib 400mg BD 24 weeks

A1: Table 66: Summary of ineligible colorectal cancer trials

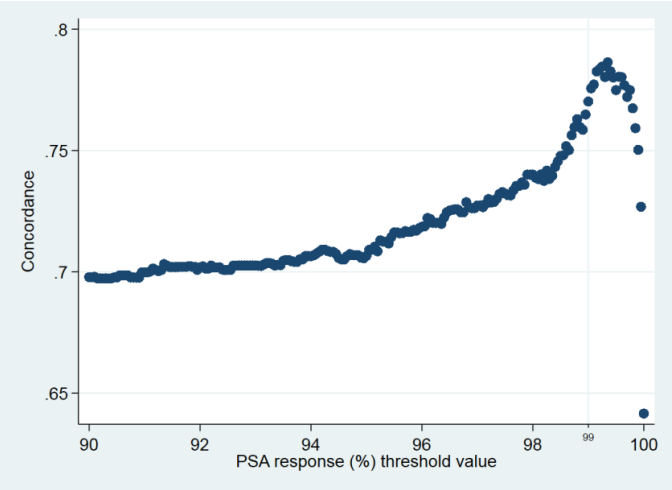
Publication	Setting	Article type	Accrual			Trial design		Treatment	Trial status	Reason for exclusion
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration		
Fenwick *et al.*2003[152]	Metastatic undergoing liver metastatectomy	Full	UK	2000 to 2002	44	2	Cox-2 inhibitor vs. Control (placebo)	Rofecoxib 25mg; minimum duration of therapy 14 days		Insufficient data
Debucoquoy *et al.*2009[328]	Localised, neo-adjuvant	Full	Belgium	2003 to 2006	35 (80)	2	RT + chemo + cox-2 vs. RT + chemo + placebo	Celecoxib 400mg BD 11-13 week duration		M0 disease
Midgley *et al.*2010[329]	Localised, adjuvant	Full	UK	2002 to 2004	2434	2	Cox-2 inhibitor vs. placebo	Rofecoxib 20mg OD	Recruitment halted due to withdrawal of rofecoxib	M0 disease
EORTC 40023 NCT00085163	Localised, adjuvant	Registry summary only	EORTC	NK	NK (1450)	3	SOC chemo + cox-2 inhibitor vs. SOC chemo + placebo	Celecoxib 400mg BD		M0 disease
Gan *et al.*2015[330]	Localised, neo-adjuvant	Abstract		2010 to 2011	150	2	3 way randomisation: Cox-2 vs. methylpred vs. placebo	Celecoxib (8mg/kg) total 10 days: 5 days pre/post op		M0 disease

A1: Table 67: Summary of ineligible PCa trials

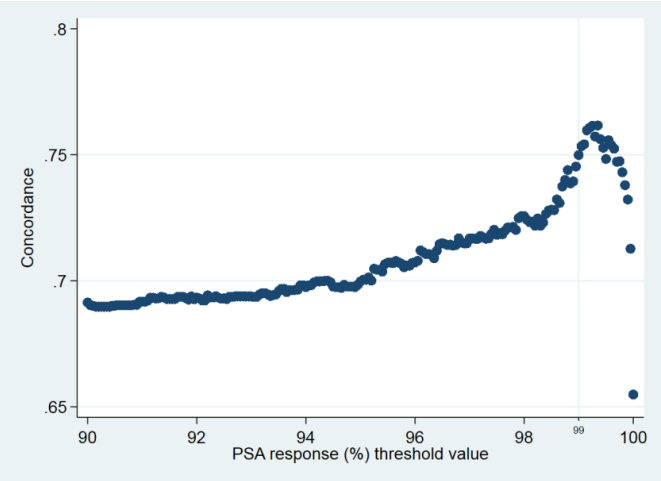
Publication	Setting	Article type	Accrual			Trial design		Treatment	Trial status	Reason for exclusion
			Country	Date	Total accrual (target)	Phase	Comparison	Cox-2 dose & duration		
Smith *et al.* 2006[96]	M0 HNPC (relapse post radical therapy)	Full	USA	2002 to 2004	78 (140)	2	Cox-2 inhibitor vs. Placebo	Celecoxib 400mg BD	Discontinued due to external safety concerns	PSA outcome data only
Antonarakis *et al.* 2009[151]	M0 Neo-adjuvant	Full	USA	2002 to 2005	64 (60)	2	Cox-2 inhibitor vs. Placebo	Celecoxib 400mg BD, 4-6 weeks prior to prostatectomy		M0 disease and translational outcomes only
Flamiatos *et al.* 2011[131]	M0, Neo-adjuvant	Abstract	USA	UN	28 (40)	2	Cox-2 inhibitor vs. Placebo	Celecoxib 400mg BD, minimum 4 weeks prior to prostatectomy	Discontinued due to cardiac AE and external safety data	M0 disease and translational outcomes only

A2: Exploring concordance for PSA response value

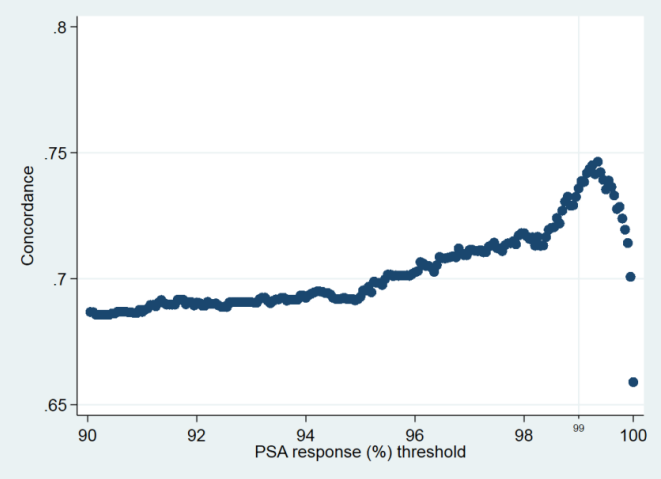
A2: Figure 38: Concordance if lower threshold ≤80% (selected)



A2: Figure 39: Concordance if lower threshold 85%



A2: Figure 40: Concordance if lower threshold 90%



A3: Analysis of PSA response in ADT alone treated group using outcome failure-free survival

A3: Table 68: Baseline multivariable FFS model for PSA response analysis

Characteristic				Overall survival	
	Category description	**n**	**Events**	**Multivariable**	**p-value***
T stage	**≤T2**	85	53	0.81 (0.54-1.20)	0.3830
	T3	380	310	1.0	
	T4	154	132	1.20 (0.90-1.59)	
	Tx	58	50	1.32 (0.89-1.97)	
Nodal stage	**N0**	228	181	1.0	0.9126
	N+	387	310	1.07 (0.80-1.43)	
	Nx	62	54	1.01 (0.66-1.54)	
Metastatic distribution	**Bone only**	428	355	3.52 (1.86-6.68)	**<0.0001**
	Distant nodal only	56	38	1.0	
	Bone & nodal or other	193	152	3.27 (1.21-2.00)	
Gleason sum score	**≤ 7**	148	103	1.0	**0.0322**
	≥ 8	445	369	1.52 (1.10-2.12)	
	Unknown	84	73	1.47 (0.95-2.28)	
Age	**≤55**	61	60	2.05 (1.35-3.13)	**0.0024**
	55-59	96	80	1.55 (1.08-2.24)	
	60-64	135	107	1.28 (0.91-1.81)	
	65-69*	181	139	1.0	
	70-74	124	95	0.85 (0.58-1.26)	
	≥ 75	80	64	1.20 (0.80-1.81)	
Performance status	**0**	495	383	1.0	**0.0007**
	1 or 2	182	162	1.56 (1.21-2.00)	
Ln (Duration of prior ADT)	***na***	545	677	0.98 (0.95-1.01)	0.1731
Ln (week-6 PSA)***	***na***	545	677	1.28 (1.21-1.37)	**<0.0001**

*For variable in overall survival model, statistically significant at 5% level shown in bold

** Median age 65 years

*** Natural logarithmic transformation

A4: Analysis of PSA response in docetaxel-treated population

A4: Table 69: Baseline overall survival model in the docetaxel-treated cohort

Characteristic				Overall survival	
	Category description	**n**	**Events**	**Multivariable**	**p-value***
T stage	**≤T2**	85	33	1.03 (0.70-1.52)	0.9911
	T3	343	137	1.0	
	T4	139	60	1.04 (0.76-1.41)	
	Tx	62	32	1.06 (0.69-1.62)	
Nodal stage	**N0**	208	73	1.0	**0.0403**
	N+	366	157	1.50 (1.09-2.06)	
	Nx	55	26	1.30 (0.83-2.04)	
Metastatic distribution	**Bone only**	392	155	1.96 (1.15- 3.34)	**0.0101**
	Distant nodal only	67	17	1.0	
	Bone & nodal or other	170	84	2.11 (1.24- 3.57)	
Gleason sum score	**≤ 7**	112	34	1.0	**0.0263**
	≥ 8	434	175	1.60 (1.10-2.32)	
	Unknown	83	47	1.64(1.04- 2.59)	
Age	**≤55**	52	22	0.89 (0.54-1.46)	0.8271
	55-59	86	39	1.05 (0.70-1.58)	
	60-64	143	57	0.91 (0.63-1.30)	
	65-69*	174	65	1.0	
	70-74	120	53	1.09 (0.75-1.58)	
	≥ 75	54	20	0.80 (0.48-1.32)	
Performance status	**0**	467	174	1.0	**0.0031**
	1 or 2	162	82	1.51 (1.16-1.98)	
Duration of prior ADT (weeks)	**Continuous**	-	-	1.06 (1.02-1.10)	**0.0014**
Ln week 6 PSA*	**Continuous**	-	-	1.14 (1.08-1.21)	**<0.0001**

*For variable in overall survival model, statistically significant at 5% level shown in bold

** Median age 65 years

*** Natural logarithmic transformation

A5: Analysis of PSA nadir in docetaxel-treated population using outcome FFS

A5: Table 70: Baseline failure-free survival model for analysis of PSA nadir

Characteristic		Failure free survival			
	Category description	n	Events	Multivariable	p-value
T stage	**≤T2**	82	56	1.26 (0.94-1.69)	
	T3	336	225	1.0	
	T4	135	93	0.96 (0.75-1.23)	
	Tx	59	45	1.08 (0.78-1.50)	0.4269
Nodal stage	**N0**	207	145	1.0	
	N+	346	225	0.91 (0.74-1.12)	
	Nx	58	49	1.35 (0.97-1.87)	0.0624
Metastatic distribution	**Bone only**	390	277	1.53 (1.06- 2.22)	
	Distant nodal only	66	33	1.0	
	Bone & nodal or other	155	109	1.43 (0.95-2.14)	0.0599
Gleason sum score	**≤ 7**	113	67	1.0	
	≥ 8	423	290	1.27 (0.97-1.66)	
	Unknown	75	62	1.21 (0.83-1.76)	0.1961
Age	**≤55**	53	41	1.06 (0.74-1.51)	
	55-59	82	60	1.07 (0.78-1.46)	
	60-64	138	98	0.84 (0.64-1.10)	
	65-69[a]	168	121	1.0	
	70-74	116	74	0.87 (0.65-1.16)	
	≥ 75	54	25	0.40 (0.26-0.62)	**<0.0001**
Performance status	**0**	453	303	1.0	
	1 or 2	159	116	1.18 (0.94-1.47)	0.1446
Duration ADT prior to randomisation	**0 week**[b]	29	19	1.05 (1.02-1.09)	**0.0048**
	≤2 weeks	77	44		
	≤4 weeks	81	51		
	≤6 weeks	104	72		
	≤8 weeks	105	77		
	≤10 weeks	104	70		
	≤12 weeks	106	81		
	≤14 weeks	5	5		
Presenting PSA[c]	**-**	611	419	1.08 (1.05-1.11)	**<0.0001**

[a] Median age 65yrs,

[b] All patients who were yet to start ADT prior to randomisation, commenced within 7 days

[c] Natural Logarithmic transformation

*For variable in overall survival model, statistically significant at 5% level shown in bold

A6: Targeted Next-generation sequencing methods

A6: Table 71: F1 Sample Processing

DNA extraction

- Pathology review H&E stained slide to ensure sample volume ≥1mm^3, nucleated cellularity ≥80% or ≥30,000 cells and 20% nucleated cells tumour derived
- Macro-dissection only if ≤20% tumour content
- Preparation of unstained FFPE sections from which DNA can be extracted (typical requirement 4x10um sections)
- DNA extraction by digestion using a proteinase K buffer followed purification (Promega Maxwell 16 Tissue LEV DNA kit)
- Quantification of DNA contraction by Picogreen fluorescence assay

Library construction

- Fragmentation of 50-200ng of dsDNA by sonication
- DNA purification performed through Agencourt AMPure XP, a buffer that selectively binds DNA fragments to paramagnetic beads allowing excess primers etc. to be washed off
- Library construction (NEBNExt kits) through addition of end repair and ligation (adding of oligonucleotides that can promote amplification)
- PCR amplification & purification
- DNA QC: Threshold >50ng of extracted DNA or 500ng of sequencing library required with mean insert size of >400 base pairs

Hybrid selection & sequencing

- Solution hybridization: the addition of complementary DNA oligonucleotides (referred to as baits) targeting the 287 cancer related exons and 47 introns of and 3549 polymorphisms (SNP). A minimum of three baits per each target exons and introns allocated (Integrated DNA Technology)
- Captured library isolated and PCR amplified (12 cycles) and quantified by qPCR (Kapa)
- Sequenced using Illumina HiSeq 2000

Bioinformatics: sequencing data processing

- Sequencing data mapped to the reference human genome (BWA aligner v0.5.9)
- Assessment of read quality made according to pre-defined algorithm that incorporates a tissue-specific estimation of whether the mutation would be expected (with reference to current published data available via COSMIC).
- Read quality quantified; reads with mapping quality of <25 and base calls with quality ≤2 excluded
- Indel detection in each exon performed through comparison of each read and imposing filters to ensure pre-defined QC metrics met e.g. the minimum mutational allele frequency required at known mutational hotspots and the neighbouring base quality
- Copy number alteration reported through combining exon coverage with SNPs against a process-matched control. Through analysis of the allele frequencies of sequenced SNPs tumour purity and copy number is estimated for each segment. Focal CNA reported if ≥6 copies reported and homozygous deletion if 0 copies, in samples with purity ≥20%
- Gene fusion detection identified by analysing read pair for which reads map to separate chromosomes (chimeric read pairs). Rearrangements annotated according to predicted function

Summarised from published methods (Frampton et al Nature Biotechnology 2016)

A6: Table 72: Ilumina TruSight Tumour T170 Panel provided by Almac Diagnostics

DNA extraction • Pathology review of FFPE block and accompanying marked H&E stained slide • Macrodissection • Dual DNA and RNA extraction using Qiagen AllPrep FFPE Kit: proteinase K containing buffer releases RNA and precipitates DNA which can be separated through centrifugation enabling separate processing of the RNA containing supernatant and DNA-containing pellet. • Separately, both RNA and DNA are incubated at 90°C aiming to reverse crosslinking • Nucleic acid concentration and purity assessed using spectrophotometric methods • Required minimum DNA concentration: 3.3ng/µL • Required minimum RNA concentration: 4.7ng/µL
Library construction • Processed using the Illumina TruSight® assay • The extracted RNA was denatured, primed with random hexamers and used to synthesise complementary DNA(cDNA) • Extracted DNA was fragmented using sonication • From this point onwards, both cDNA and DNA were processed together • Initial library construction achieve through ligation of adaptors (oligonucleotides that can promote amplification), purification and then amplification
Hybrid selection & sequencing • Hybridisation: addition of a custom pool of oligonucleotides specific to the targeted regions of interest • Purification • PCR amplification using primers binding regions that flank targeted regions of interest • Quantification & QC assessment using fluorometric methods • Bead-based normalisation: DNA is bound to normalisation beads and then eluted off the beads at a similar concentration, allowing even library quantification required prior to loading sequencing machine • Sequenced using Illumina NextSeq
Bioinformatics: sequencing data processing • Sequencing data aligned to the Reference Human Genome • Analysed using the TST170 application on BaseSpace; this is an online resource supporting Illumina products that enables storage and analysis of sequencing data using a variety of applications. • QC assessment performed as part of BaseSpace application • Custom script developed by Almac in conjunction with Clovis' pre-defined enrolment eligibility criteria applied to processed files outputted via BaseSpace

A7: Comparative Targeted Next-generation sequencing gene lists

A7: Figure 41: Foundation One Gene List

ABL1	BRCA1	CREBBP	FANCD2	GID4	KDM5C	MUTYH	PIK3R1	RUNX1	TET2
ABL2	BRCA2	CRKL	FANCE	GLI1	KDM6A	MYB	PIK3R2	RUNX1T1	TGFBR2
ACVR1B	BRD4	CRLF2	FANCF	GNA11	KDR	MYC	PLCG2	SDHA	TMPRSS2
AKT1	BRIP1	CSF1R	FANCG	GNA13	KEAP1	MYCL	PMS2	SDHB	TNFAIP3
AKT2	BTG1	CTCF	FANCL	GNAQ	KEL	MYCN	POLD1	SDHC	TNFRSF14
AKT3	BTK	CTNNA1	FAS	GNAS	KIT	MYD88	POLE	SDHD	TOP1
ALK	C11orf30	CTNNB1	FAT1	GPR124	KLHL6	NF1	PPP2R1A	SETD2	TOP2A
AMER1	CARD11	CUL3	FBXW7	GRIN2A	KMT2A	NF2	PRDM1	SF3B1	TP53
APC	CBFB	CYLD	FGF10	GRM3	KMT2C	NFE2L2	PREX2	SLIT2	TSC1
AR	CBL	DAXX	FGF14	GSK3B	KMT2D	NFKBIA	PRKAR1A	SMAD2	TSC2
ARAF	CCND1	DDR2	FGF19	H3F3A	KRAS	NKX2-1	PRKCI	SMAD3	TSHR
ARFRP1	CCND2	DICER1	FGF23	HGF	LMO1	NOTCH1	PRKDC	SMAD4	U2AF1
ARID1A	CCND3	DNMT3A	FGF3	HNF1A	LRP1B	NOTCH2	PRSS8	SMARCA4	VEGFA
ARID1B	CCNE1	DOT1L	FGF4	HRAS	LYN	NOTCH3	PTCH1	SMARCB1	VHL
ARID2	CD274	EGFR	FGF6	HSD3B1	LZTR1	NPM1	PTEN	SMO	WISP3
ASXL1	CD79A	EP300	FGFR1	HSP90AA1	MAGI2	NRAS	PTPN11	SNCAIP	WT1
ATM	CD79B	EPHA3	FGFR2	IDH1	MAP2K1	NSD1	QKI	SOCS1	XPO1
ATR	CDC73	EPHA5	FGFR3	IDH2	MAP2K2	NTRK1	RAC1	SOX10	ZBTB2
ATRX	CDH1	EPHA7	FGFR4	IGF1R	MAP2K4	NTRK2	Rad50	SOX2	ZNF217
AURKA	CDK12	EPHB1	FH	IGF2	MAP3K1	NTRK3	Rad51	SOX9	ZNF703
AURKB	CDK4	ERBB2	FLCN	IKBKE	MCL1	NUP93	Rad51B	SPEN	
AXIN1	CDK6	ERBB3	FLT1	IKZF1	MDM2	PAK3	Rad51C	SPOP	
AXL	CDK8	ERBB4	FLT3	IL7R	MDM4	PALB2	Rad51D	SPTA1	
BAP1	CDKN1A	ERG	FLT4	INHBA	MED12	PARK2	Rad54L	SRC	
BARD1	CDKN1B	ERRFI1	FOXL2	INPP4B	MEF2B	PAX5	RAF1	STAG2	
BCL2	CDKN2A	ESR1	FOXP1	IRF2	MEN1	PBRM1	RANBP2	STAT3	
BCL2L1	CDKN2B	ETV1	FRS2	IRF4	MET	PDCD1LG2	RARA	STAT4	
BCL2L2	CDKN2C	ETV4	FUBP1	IRS2	MITF	PDGFRA	RB1	STK11	
BCL6	CEBPA	ETV5	GABRA6	JAK1	MLH1	PDGFRB	RBM10	SUFU	
BCOR	CHD2	ETV6	GATA1	JAK2	MPL	PDK1	RET	SYK	
BCORL1	CHD4	EZH2	GATA2	JAK3	MRE11A	PIK3C2B	RICTOR	TAF1	
BCR	CHEK1	FAM46C	GATA3	JUN	MSH2	PIK3CA	RNF43	TBX3	
BLM	CHEK2	FANCA	GATA4	KAT6A	MSH6	PIK3CB	ROS1	TERC	
BRAF	CIC	FANCC	GATA6	KDM5A	MTOR	PIK3CG	RPTOR	TERT	

Key
Present in F1
Selected HRD Gene
Unique to t170

A7: Figure 42: TruSight T170 (t170) Gene List (Almac Diagnostics)

ABL1	CARD11	EGFR	FGF1	FOXL2	MCL1	NOTCH2	PPP2R2A	STK11
AKT1	CCND1	EML4	FGF10	GEN1	MDM2	NOTCH3	PTCH1	TERT
AKT2	CCND2	EP300	FGF14	GNA11	MDM4	NPM1	PTEN	TET2
AKT3	CCND3	ERBB2	FGF19	GNAQ	MET	NRAS	PTPN11	TFRC
ALK	CCNE1	ERBB3	FGF2	GNAS	MLH1	NRG1	Rad51	TMPRSS2
APC	CD79A	ERBB4	FGF23	HNF1A	MLLT3	NTRK1	Rad51B	TP53
AR	CD79B	ERCC1	FGF3	HRAS	MPL	NTRK2	Rad51C	TSC1
ARID1A	CDH1	ERCC2	FGF4	IDH1	MRE11A	NTRK3	Rad51D	TSC2
ATM	CDK12	ERG	FGF5	IDH2	MSH2	PALB2	Rad54L	VHL
ATR	CDK4	ESR1	FGF6	INPP4B	MSH3	PAX3	RAF1	XRCC2
AXL	CDK6	ETS1	FGF7	JAK2	MSH6	PAX7	RB1	
BAP1	CDKN2A	ETV1	FGF8	JAK3	MTOR	PDGFRA	RET	
BARD1	CEBPA	ETV4	FGF9	KDR	MUTYH	PDGFRB	RICTOR	
BCL2	CHEK1	ETV5	FGFR1	KIF5B	MYC	PIK3CA	ROS1	
BCL6	CHEK2	EWSR1	FGFR2	KIT	MYCL1	PIK3CB	RPS6KB1	
BRAF	CREBBP	EZH2	FGFR3	KMT2A	MYCN	PIK3CD	SLX4	
BRCA1	CSF1R	FAM175A	FGFR4	KRAS	MYD88	PIK3CG	SMAD4	
BRCA2	CTNNB1	FANCI	FLI1	LAMP1	NBN	PIK3R1	SMARCB1	
BRIP1	DDR2	FANCL	FLT1	MAP2K1	NF1	PMS2	SMO	
BTK	DNMT3A	FBXW7	FLT3	MAP2K2	NOTCH1	PPARG	SRC	

Key
Selected HRD Gene
Also present in F1
Unique to t170

A7: Figure 43: Color Genomics Gene List (germline analysis)

APC	BRCA1	CHEK2	MLH1	PMS2	SMAD4
ATM	BRCA2	CDKN2A	MSH2	POLD1	STK11
BAP1	BRIP1	EPCAM	MUTYH	PTEN	TP53
BARD1	CDH1	GREM1	NBN	Rad51C	
BMPR1A	CDK4	MITF	PALB2	Rad51D	

Key
Selected HRD Gene

A8: Sites contributing samples to each sequencing analysis

A8: Table 73: Sites contributing samples to analysis one (F1)

Site name	Site Type	Sample number	Sequenced	Success rate (%)
Velindre, Cardiff	Tertiary	43	32	76%
Singleton, Swansea	Tertiary	36	16	44%
Stepping Hill, Stockport	DGH	21	6	29%
Royal United Hosp Bath	DGH	19	4	21%
Ipswich Hosp	DGH	13	12	92%
Huddersfield	DGH	13	10	77%
Broomfield Hosp, Chelmsford	DGH	11	11	100%
Bristol	Tertiary	9	6	67%
Royal Devon & Exeter	Tertiary	7	6	86%
Raigmore, Inverness	DGH	5	5	100%
Blackburn, East Lanc	DGH	3	3	100%
King's Mill	DGH	2	2	100%
Queen Alexandra, Portsmouth	Tertiary	2	1	50%
Bolton	DGH	2	1	50%
Hereford	DGH	1	1	100%
TOTALS				
Teritary sites	5			
DGH sites	10			
Total number of sites	15			
Samples	186			
Sequenced	115			
Success rate	61%			

	≥5 samples
	<5 samples
	below average sequencing success rate

A8: Table 74: Sites contributing samples to analysis two (t170)

Site name	Site Type	Sample number	Sequenced	Success rate (%)
Torbay District General Hospital	DGH	11	11	100%
Pilgrim Hospital	DGH	7	5	71%
York Teaching Hospital	Tertiary	7	2	29%
Royal Devon and Exeter Hospital	Tertiary	6	5	83%
Guy's Hospital (London)	Tertiary	6	5	83%
Lister Hospital	DGH	5	5	100%
Darlington Memorial Hospital	DGH	5	3	60%
Barnet General Hospital	DGH	5	3	60%
Velindre Hospital	Tertiary	4	4	100%
Lincoln County Hospital	DGH	4	4	100%
Royal Albert Edward Infirmary	DGH	4	3	75%
University College Hospital	Tertiary	3	3	100%
Burnley General Hospital	DGH	3	3	100%
Royal Bournemouth Hospital	DGH	3	2	67%
Musgrove Park Hospital	DGH	3	2	67%
Weston General Hospital	DGH	3	2	67%
Broomfield Hospital	DGH	2	2	100%
Ipswich Hospital	DGH	2	2	100%
Worthing Hospital	DGH	2	2	100%
North Middlesex Hospital	DGH	2	2	100%
Singleton Hospital	Tertiary	2	1	50%
Southend University Hospital	DGH	2	1	50%
Royal Bolton Hospital	DGH	2	1	50%
Raigmore Hospital	DGH	2	1	50%
Southampton General Hospital	Tertiary	1	1	100%
Yeovil District Hospital	DGH	1	1	100%
James Cook University Hospital	Tertiary	1	0	0%
Essex County Hospital	DGH	1	0	0%
TOTALS				
Teritary sites	8			
DGH sites	20			
Total number of sites	28			
Samples	99			
Sequenced	76			
Success rate	77%			

	≥5 samples
	<5 samples
	below average sequencing success rate

A9: Treatment allocation for patients sampled in each sequencing analysis

A9: Table 75: Population included in analysis one by research arm

STAMPEDE Treatment allocation	
Arm A: Control (SOC)	76 (41%)
Arm B: SOC+ zoledronic acid	8 (4%)
Arm C: SOC + docetaxel	7 (4%)
Arm E: SOC + docetaxel + zoledronic acid	6 (3%)
Arm G: SOC + abiraterone	21 (11%)
Arm H: SOC+ M1|RT	38 (20%)
Arm K: SOC + metformin	31 (17%)

A9: Table 76: Population included in analysis two by research arm

STAMPEDE Treatment allocation	
Arm A: Control (SOC)	32
Arm H: SOC + Prostate RT	47
Arm K: SOC + Metformin	21

A10: Evidence of pre-analytical variability between sequencing providers

A10: Table 77: Comparative DNA concentration estimates

#	FM assessment ng/ul	Almac assessment ng/ul	Comparison (%)	Almac QC
1	4.42	0	0.00	Fail
2	5.079	0	0.00	Fail
3	4.992	2.7	54.09	Fail
4	8.652	6.94	80.21	Pass
5	15.781	15.6	98.85	Pass
6	11.088	11	99.21	Pass
7	245.036	248	101.21	Pass
8	61.254	63.8	104.16	Pass
9	41.851	45	107.52	Pass
10	22.935	26.6	115.98	Pass
11	10.544	12.6	119.50	Pass
12	49.071	60	122.27	Pass
13	21.037	26.2	124.54	Pass
14	24.546	30.6	124.66	Pass
15	7.467	9.4	125.89	Pass
16	58.681	74.2	126.45	Pass
17	49.059	65.8	134.12	Pass
18	191.494	326	170.24	Pass
19	18.761	35.6	189.76	Pass
20	14.077	43.4	308.30	Pass

Key: QC, quality control; Almac, Almac diagnostics; FM, Foundation Medicine. Green shading indicates where results are within 10%

Appendix B: Summary of ongoing PARPi trials and other therapies in HRD Prostate cancer

AB: Table 78: PARPi trials in prostate cancer -1

Agent	Registration Acronym	Phase	Patient group	Biomarker	Treatment details	Primary outcome measures	Status
Olaparib	NCT01682772 TOPARP	II	mCRPC	Mandatory biopsies tNGS BRCA1, BRCA2, ATM, + other HRD genes	• ADT + olaparib	ORR	TOPARP-A: reported TOPARP-B: ongoing
Olaparib	NCT02987543 PROfound	III	mCRPC (post progression on abi or enza)	Tumour HRD testing: broader panel recruited, powered on subset	• ADT + olaparib • ADT + abi or enza	rPFS in BRCA/ATM	Recruiting Est accrual: 340 Est report date: Feb 2021
Olaparib	NCT03012321	II	mCRPC	BRCA1/BRCA2/ATM mutations eligible for randomisation between arms 1-3. Non-BRCA/ATM HRD mutant cases eligible for arm 4 (one or more mutations in 17 HRD genes)	• Arm 1: ADT + abi +pred • Arm 2: ADT + olaparib • Arm 3: ADT + abi + pred + olaparib • Arm 4: ADT + olaparib	PFS at 2 years	Recruiting Target accrual: 70 Est completion date: Jan 2022
Olaparib	NCT03047135	II	High risk biochemical relapsed	HRD panel of 20 genes Two stage design (similar to TOPARP) to evaluate different biomarker enrichment	• ADT+ olaparib	PSA RR	Target accrual: 50 Est report: 2022
Olaparib	NCT02861573 KEYNOTE-365	Ib/II	mCRPC	unselected	• Cohort 1: Pembro + olaparib • Cohort 2: Pembro + docetaxel + olaparib • Cohort 3: Pembro + enza	Safety and PSA response (>50%)	Recruiting Target accrual 70 per cohort (210) Est completion: April 2020
Olaparib	NCT03434158 IMANOL	II	mCRPC	PR or SD post docetaxel HRD positive (pathogenic mutation in any HRD or MMR gene)	• ADT + Olaparib	rPFS	Recruiting Target accrual: 27 Est report: Nov 2019

AB: Table 79: PARPi trials in prostate cancer -2

Agent	Registration Acronym	Phase	Patient group	Biomarker	Treatment details	Primary outcome measures	Status
Rucaparib	NCT02952534 TRITON2	II	mCRPC	**3 cohorts:** A: BRCA1/2 or ATM with measurable disease B: BRCA1/2 or ATM C: non BRCA/ATM HRD (mutation in one or more 15 HRD genes)	• ADT+ rucaparib	ORR PSA response	Recruiting Target accrual: 160 Est report date: April 2020
Rucaparib	NCT02975934 TRITON3	III	mCRPC (bone)	BRCA1/2 or ATM mutant somatic or germline on local or centralised testing	• ADT + rucaparib • ADT + physicians choice (docetaxel/abiraterone/ enzalutamide)	rPFS	Recruiting Accrual target: 400 Est report date: Feb 2022
Rucaparib	NCT03413995 TRIUMPH	II	Metastatic hormone-sensitive	Pathological germline mutation in one or more of 20 HRD on a clinically accredited test	• Rucaparib alone (ADT not being given)	PSA response	Start date: May 2018 Target accrual: 30 Est report date: May 2020
Rucaparib	NCT03442556 PLATI-PARP	II	mCRPC	BRCA1/2 or ATM	• Carboplatin, Docetaxel & Rucaparib	rPFS	Target accrual 20 Start May 2018 Completion May 2024
Rucaparib	NCT03338790 CheckMate 9KD	II		Unselected but mandatory tumour testing – likely subgroup?	• Nivolumab+ rucaparib • Nivolumab, + docetaxel + pred • Nivolumab + enza	ORR	Recruiting Completion Feb 2020

AB: Table 80: PARPi trials in prostate cancer -3

Agent	Registration Acronym	Phase	Patient group	Biomarker	Treatment details	Primary outcome measures	Status
Niraparib	NCT02854436 Galahad	II	mCRPC	Tumour positive for DNA repair abnormalities (company assay)	• ADT + Niraparib	ORR	Recruiting Accrual target: 160 Est report date: Oct 2019
Niraparib	NCT03431350	Ib/II	mCRPC	Cohort 1A: BM positive (HRD) 1B: BM negative	• ADT + Niraparib • ADT + Niraparib + anti-PD1 (JNJ-63723283) 240mg • ADT + Niraparib + anti-PD1 480mg •	Safety & ORR	Target accrual: 60 Started March 2018 July 2019
Niraparib	NCT02924766 BEDIVERE	I/Ib	mCRPC	unselected	• ADT + Niraparib +apalutamide • ADT + Niraparib + abi + pred	MTD & Safety	Recruiting Target accrual: 60 Est completion date: May 2018
Talazoparib	NCT03148795	II	mCRPC	HRD as defined by central panel + germline testing	• ADT+Talazoparib	ORR	Recruiting Target accrual: 100 Est completion ~2021
Talazoparib	NCT03395197 TALAPRO-2	III	mCRPC	HRD as defined by central panel + germline testing: Fresh biopsy required if archival tissue not sufficient	• ADT+ Talazoparib + abi or enza • ADT + placebo + physicians choice of abi or enza	Safety (part 1) rPFS (part 2)	Recruiting Started Dec 2017 Target accrual: 444 Est completion: March 2024

Appendix C: STAMPEDE protocol version 17.0 and associated documents

Link to STAMPEDE Protocol version 17.0 -Describes the Biomarker screening pilot
http://www.stampedetrial.org/media/1841/01-stampede_protocol_v17_clean.pdf

Link to STAMPEDE Sample Handling Manual
http://www.stampedetrial.org/media/1742/stampede_sample_collection_handling_manualv5.pdf

Appendix D: Prospero Systematic Review Protocol

Appendix E: Data release approval letter

Appendix F: Biomarker Development Protocol

Appendix G: Confirmation of Ethical approval

Appendix H: Foundation One Sampling requirements

Appendix I: Relevant publication

PROSPERO International prospective register of systematic reviews

Use of Cox-2 inhibitors in the treatment of cancer: a systematic review of the evidence

Clare Gilson, Sarah Burdett

Citation

Clare Gilson, Sarah Burdett. Use of Cox-2 inhibitors in the treatment of cancer: a systematic review of the evidence. PROSPERO 2016:CRD42016041743 Available from
http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42016041743

Review question(s)

Can the addition of Cox-2 inhibitors improve outcomes in cancer?

Searches

Electronic databases:

MEDLINE 1996-2016

EMBASE 1996-2016

Cochrane Central Review of Controlled Trials (CENTRAL) 1996-present (via OVID)

Trial registers:

ClinicalTrials.gov

Conference proceedings:

Proceedings of the American Society of Clinical Oncology (ASCO) 2004-2016

Proceedings of the European Society of Medical Oncology (ESMO) 1996-2016

Proceedings of the European Cancer Conference Organization (ECCO) 1996-2016

Searching for trials focusing on six tumour sites/types of interest (Gastrointestinal malignancies, non-small cell lung, breast, prostate, head and neck, myeloma).

Types of study to be included

Phase II and III randomised controlled trials will be included if they have aimed to evaluate Cox-2 inhibitors alone or in combination. In order to provide historical context, particularly as many later stage trials were terminated due to concerns regarding cardiovascular toxicity, non-randomised phase II trials will also be reviewed separately.

Condition or domain being studied

Cancer.

Trials will be reviewed by tumour site focusing on six tumour sites/types of interest:

• Gastrointestinal malignancies (upper and lower gastrointestinal tract, including colorectal and hepatobiliary)

• Non-small cell lung

• Breast

• Prostate

• Head and neck

• Myeloma

Participants/ population
Patients with cancers in whom Cox-2 inhibitors are being evaluated alone or in combination in any disease setting: including neo-adjuvant (prior to radical surgery or radiotherapy), adjuvant (following radical therapy) and palliative (i.e. treatment for metastatic disease).

Intervention(s), exposure(s)
Cox-2 inhibitor should be given alone or in combination with additional research treatment or current standard-of-care.

Comparator(s)/ control
In controlled trials, this group should receive standard-of-care treatment or placebo.

Context
Neo-adjuvant (prior to radical surgery or radiotherapy), adjuvant (following radical therapy) and palliative (i.e. treatment for metastatic disease).

Outcome(s)
Primary outcomes
Progression-free survival.

Time to progression or death.

Secondary outcomes
Overall survival

Disease-free survival

Response rate

Cardiovascular toxicity

Translational endpoints are of interest although are not amenable to analysis.

Data extraction, (selection and coding)
Data on patient characteristics, interventions and outcomes will be extracted from publications and presentations into pre-designed forms. Where insufficient data are available from publications, it may be sought directly from investigators.

Risk of bias (quality) assessment
These will be carried out using the Cochrane Risk of Bias Tool both for individual trials and, if it is possible to proceed to undertake a meta-analysis, this will be assessed for overall meta-analysis for the primary outcome of overall survival.

Strategy for data synthesis
If sufficient data is available having undertaken a systemic review, a meta-analysis will performed of time-to-event outcomes following the extraction of hazard ratio (HR) and associated statistics from the trial reports. Where not reported, they will be estimated from the Kaplan-Meier curves or other summary statistics using published methods. Where insufficient data are available, supplementary data may be sought directly from the trial investigators. If sufficient data is available this will be looked at across all trials and within sub-groups by tumour site. If sufficient data is available this will be looked at across all trials and within sub-groups by tumour site.

Analysis of subgroups or subsets
Cancer site focusing on five sites/types of interest.

Setting: Neo-adjuvant, adjuvant, metastatic.

Should sufficient data be available a planned analysis of the metastatic populations across common tumour types will be performed.

Dissemination plans
The results will be submitted for presentation at an international cancer meeting and for publication in a peer reviewed journal.

Contact details for further information
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Organisational affiliation of the review
MRC Clinical Trials Unit at UCL

Review team
Dr Clare Gilson, MRC CTU at UCL
Ms Sarah Burdett, MRC CTU at UCL

Anticipated or actual start date
04 July 2016

Anticipated completion date
05 September 2016

Funding sources/sponsors
MRC CTU at UCL

Conflicts of interest
None known

Language
English

Country
England

Subject index terms status
Subject indexing assigned by CRD

Subject index terms

Anti-Inflammatory Agents, Non-Steroidal; Cyclooxygenase 2 Inhibitors; Humans; Neoplasms

Stage of review
Ongoing

Date of registration in PROSPERO
06 July 2016

Date of publication of this revision
06 July 2016

Stage of review at time of this submission	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Cox-2 inhibitors in the treatment of cancer: search strategy

Medline Search Strategy	Medline non-randomised Search strategy	Central search strategy	Embase search strategy
1 randomized controlled trial (pt) 2 controlled clinical trial (pt) 3 randomized (ab, ti) 4 placebo (ab, ti) 5 clinical trials as topic.sh 6 randomly (ab, ti) 7 trial (ab, ti) 8 1 or 2or 3 or 4 or 5 or 6 or 7 9 exp neoplasms 10 cancer in AB or cancer in TI 11 9 or 10 12 rofecoxib in AB, TI 13 vioxx in AB, TI 14 celecoxib in AB, TI 15 celebrex in AB, TI 16 etoricoxib in AB, TI 17 arcoxia in AB, TI 18 valdecoxib in AB, TI 19 bextra in AB, TI 20 etodolac in AB, TI 21 lodine in AB, TI 22 meloxicam in AB, TI 23 mobic in AB, TI 24 parecoxib in AB, TI 25 dynastat in AB, TI 26 lumiracoxib in AB, TI 27 prexige in AB, TI *28 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27* *29 8 and 11 and 28*	1 clinical trials as topic.sh 2 trial (ab, ti) 3 1 or 2 4 exp neoplasms 5 cancer in AB or cancer in TI 6 4 or 5 7 rofecoxib in AB, TI 8 vioxx in AB, TI 9 celecoxib in AB, TI 10 celebrex in AB, TI 11 etoricoxib in AB, TI 12 arcoxia in AB, TI 13 valdecoxib in AB, TI 14 bextra in AB, TI 15 etodolac in AB, TI 16 lodine in AB, TI 17 meloxicam in AB, TI 18 mobic in AB, TI 19 parecoxib in AB, TI 20 dynastat in AB, TI 21 lumiracoxib in AB, TI 22 prexige in AB, TI 23 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 24 8 and 11 and 28	1 Cancer 2 Rofecoxib 3 Vioxx 4 Celebrex 5 Etoricoxib 6 Arcoxia 7 Valdecoxib 8 Bextra 9 Etodolac 10 Iodine 11 Mobic 12 Parecoxib 13 Lumiracoxib 14 Prexige 15 cox-2 16 Celecoxib 17 #2 to #16 (or) 18 #1 and #17	1 neoplasm 2 cancer.ti,ab. 3 1 or 2 4 crossover procedure 5 double blind procedure 6 randomized controlled trial 7 single blind procedure 8 random.mp 9 factorial.mp. 10 (crossover* or cross over* or cross-over*) mp 11 placebo*mp 12 (double* adj blind*).mp. 13 (singl* adj blind).mp. 14 assign*.mp. 15 allocat*.mp. 16 volunteer*.mp. 17 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 18 rofecoxib.ti,ab. 19 vioxx.ti,ab. 20 celecoxib.ti,ab. 21 celebrex.ti,ab. 22 etoricoxib.ti,ab. 23 arcoxia.ti,ab. 24 valdecoxib.ti,ab. 25 bextra.ti,ab. 26 etodolac.ti,ab. 27 eccoxalac.ti,ab. 28 etopan.ti,ab. 29 lodine.ti,ab. 30 meloxicam.ti,ab. 31 mobic.ti,ab. 32 parecoxib.ti,ab. 33 dynastat.ti,ab. 34 lumiracoxib.ti,ab. 35 prexige.ti,ab. 36 *18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35* 37 *3 and 17 and 36*

Clare Gilson and Robert Laing
MRC Clinical Trials Unit at UCL
Institute of Clinical Trials & Methodology
Aviation House
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London
WC2B 6NH

08th May 2017

Dear Dr Clare Gilson and Dr Robert Laing,

Data release request for STAMPEDE: Investigating the prognostic importance of PSA nadir in men with hormone-naïve prostate cancer receiving first-line ADT and docetaxel

Thank you for your application to UCL for the release of data from the STAMPEDE trial.

The application has been reviewed by the STAMPEDE Trial Management Group and STAMPEDE Trial Steering Committee, and I am pleased to inform you that both oversight groups have approved the application, in principle.

If you have any questions please don't hesitate to contact us.

Yours sincerely,

On behalf of the STAMPEDE Trial Management Group

CC Nick James, Matthew Sydes

Biomarker Development using STAMPEDE Archival tissue samples

Protocol v.1.0

18th March 2016

Chief Investigator Signature:

Date: 24-MARCH-2016

1 GENERAL INFORMATION

This translational protocol describes a sub-study of STAMPEDE coordinated by the MRC CTU at UCL which is a registered trial with the ClinicalTrials.gov Clinical Trials Register, where it is identified as NCT00268476.

CHIEF INVESTIGATOR

Dr Clare Gilson
Clinical Research Fellow
MRC CTU at UCL

CO-INVESTIGATORS

Mr Matthew Sydes
Reader in Clinical Trials and Methodology, MRC CTU at UCL

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Dr Simon Chowdhury
Consultant Oncologist, Guys and St Thomas' Hospital

Professor Malcolm Mason
Professor of Clinical Oncology, Cardiff University
Chair of STAMPEDE Biological Research Group

Professor Nicholas James
Professor of Clinical Oncology, University of Warwick
STAMPEDE Chief Investigator

INDUSTRY COLLABORATORS

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Almac Diagnostics
19 Seagoe Industrial Estate, Northern Ireland, UK

Dr Mitch Raponi
Clovis Oncology in partnership with Foundation Medicine
Foundation Medicine
150 Second, Cambridge, MA, USA

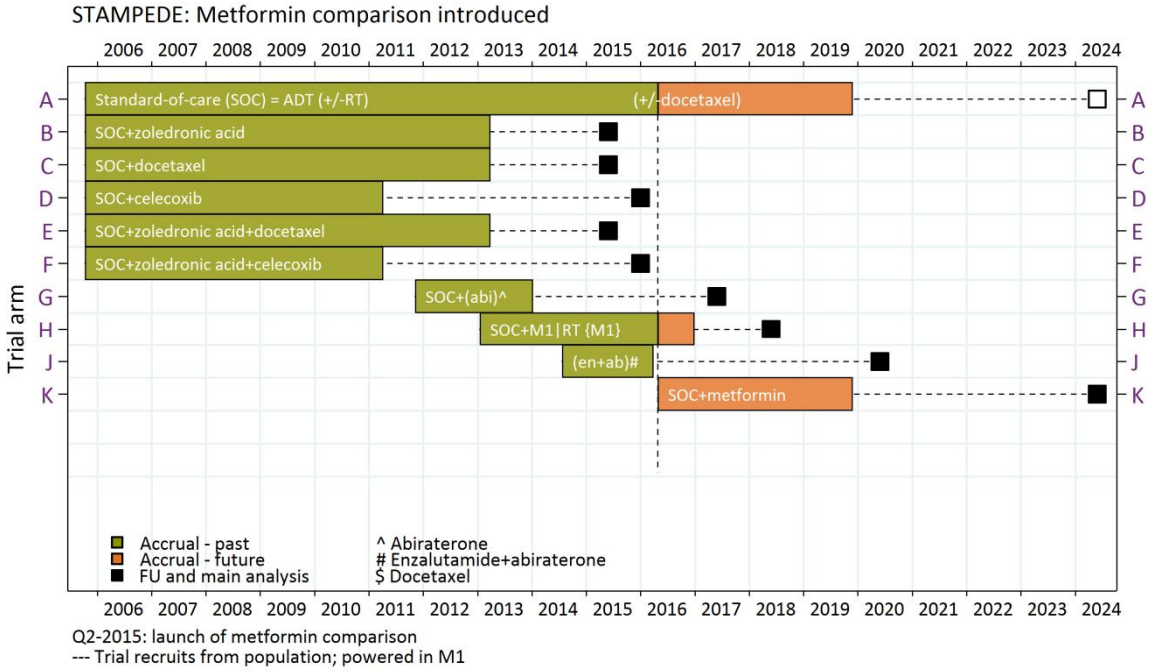
2 SUMMARY

Prostate cancer is a highly heterogeneous disease with a growing number of treatment options. Yet despite increased understanding of tumour biology, prostate cancer management lags behind that of other cancers due to the lack of predictive biomarkers.

Integration of clinical and translational research is urgently needed to develop biomarkers able to inform treatment selection and provide a rationale for additional targeted strategies.

Systematic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE) is an ongoing multi-centre randomised controlled trial for men with locally-advanced or metastatic prostate cancer. Using a multi-arm, multi-stage (MAMS) platform design STAMPEDE will evaluate at least 9 treatment strategies. Analysis of the archival tumour tissue from randomised comparisons within STAMPEDE offers a unique opportunity to support biomarker development through associated translational sub-studies.

Figure 1: Treatments evaluated over time in the MAMS STAMPEDE platform



3 BACKGROUND

STAMPEDE is a MAMS trial platform that recruits men with locally-advanced or metastatic prostate cancer who are commencing long-term androgen deprivation therapy (ADT) for the first time. The trial evaluates whether adding additional treatment strategies to the current 1st line standard-of-care treatments improves overall survival.

Since opening in 2005, STAMPEDE has recruited over 8000 patients and will evaluate at least 9 treatment strategies (see figure 1). Each research comparison is compared against a shared control arm and is conducted in a number of stages; a Pilot/safety phase, Activity Stages and final Efficacy Stage. The primary outcome measure is failure-free survival (FFS) for each Activity Stage with the final Efficacy Stage based on overall survival (OS).

The primary results of the "original comparisons" have demonstrated an overall survival benefit when docetaxel chemotherapy is added to ADT which has changed practice. The STAMPEDE cohort includes men with both metastatic (60%) and locally advanced disease (40%). Overall, the addition of docetaxel improved survival by 10 months, from 71 to 80 months (HR 0.78; 0.66-0.93; p=0.006). No evidence of heterogeneity was seen in a planned sub-group analysis by metastatic status. Yet survival data remains immature in the non-metastatic (M0) sub-group as these men have a better prognosis. In M0 disease docetaxel was shown to improve disease control rates and the effect on prostate cancer-specific survival remains consistent[1].

In response to the results of STAMPEDE and CHAARTED both of which were included in a simultaneously published meta-analysis, UK practice is changing[1]. This is supported by a NHS England rapid evidence review which now recommends docetaxel for newly diagnosed hormone-naïve metastatic prostate cancer[2]. The standard-of-care within the trial has also been updated accordingly.

All men joining STAMPEDE have been asked to provide consent for the use of their tissue samples obtained at prostate biopsy or following prostate surgery for additional research. Over 2000 patients contributed to the randomised comparison of docetaxel (given alone and in combination with zoledronic acid). Analysis of archival tissue belonging to this cohort provides a unique opportunity to develop prognostic and predictive biomarkers relating to the new standard-of-care treatment. As practice has now changed, this randomised comparison will not be replicated and therefore represents a valuable translational resource to support biomarker development.

4 STUDY AIMS

The aim of this translational programme of work is to develop biomarkers capable of stratifying men at the point at which long-term ADT is started. The following questions are relevant to this population:

- Can prognostic biomarkers stratify locally-advanced prostate cancers and predict benefit from adjuvant docetaxel?
- Can predictive biomarkers identify prostate cancers that benefit less from docetaxel and therefore identify in whom alternative treatment strategies should be evaluated?
- Can biomarkers of DNA repair deficiency hypothesised to predict response to alternative treatment strategies have clinical utility in prostate core biopsy tissue?
- What is the prevalence of 'targetable' genetic defects in newly diagnosed metastatic disease?

5 STUDY DESIGN

This pilot study will be composed of three analyses each evaluating a biomarker of interest.

5.1 ANALYSIS 1: CLINICAL VALIDATION OF A PROGNOSTIC BIOMARKER IN NON-METASTATIC DISEASE

In collaboration with Professor Richard Kennedy (Queen's University Belfast/Almac Diagnostics) a prognostic signature will be evaluated in non-metastatic (M0) disease. Preliminary work using unsupervised clustering has identified two major molecular subsets within M0 disease. A 70-gene signature can distinguish these two cohorts and has been shown to be prognostic (Time to metastasis HR=6.32; Time to PSA failure HR=3.76). We will aim to validate this prognostic biomarker within a sub-set of M0 patients within the randomised docetaxel cohort in STAMPEDE. Through comparing samples from both control and docetaxel treated patients the predictive effect of this biomarker will be investigated. If shown to be capable of identifying cancers at high risk of relapse, this biomarker could inform decisions as to who requires adjuvant docetaxel.

5.1.1 ANALYSIS OBJECTIVES

- The evaluate the feasibility of using the Prostate Prognostic biomarker as a molecular stratifier in the high-risk locally advanced population in STAMPEDE
- To determine the prevalence of the poor prognostic population as identified by this assay
- To correlate with clinical outcome data (failure-free survival and overall survival) to determine if the poor prognostic group benefit from docetaxel treatment

5.2 ANALYSIS 2: EVALUATING GENOMIC LOSS-OF-HETEROZYGOSITY (GENOMIC LOH) AS A POTENTIAL PREDICTIVE BIOMARKER

In collaboration with Clovis Oncology, genomic LOH will be assessed as potential predictive biomarker of sensitivity to PARP inhibition. Clovis Oncology in partnership with Foundation Medicine have developed this assays of genomic scarring, specifically genome-wide loss of heterozygosity (LOH), which is the phenotypic result of homologous recombination deficiency (HRD). Loss of heterozygosity occurs when one copy of a heterozygous region of DNA is lost and the other is retained. Importantly this can result from alterations in multiple genes, including from those for which the mechanism is currently unknown[3]. An assessment of genomic LOH is used to provide a HRD score which has been shown to be well correlated with homologous recombination, a major pathway of DNA repair[4]. Essentially this provides a functional test of DNA repair capability. This has been validated in ovarian cancer within the ARIEL 2 trial and shown to predict response to the PARP inhibitor Rucaparib. This project will aim to evaluate the clinical utility of this potential predictive biomarker in prostate cancer in order to evaluate a PARP inhibitor in an appropriate biomarker defined group.

5.2.1 ANALYSIS OBJECTIVES

- To determine the feasibility of assessing genomic LOH in prostate core biopsy tissue
- To determine the prevalence of DNA repair deficiency as determined by high genomic LOH in men with newly diagnosed metastatic disease

- To correlate with clinical outcome data in order to characterise the biomarker defined groups

5.3 ANALYSIS 3: ASSESSING THE IMMUNE RESPONSE TO DNA DAMAGE USING A GENE-EXPRESSION SIGNATURE- DNA DAMAGE RESPONSE DEFICIENCY (DDRD)

Originally developed in a breast cancer cohort, this 44-gene signature predicts response to DNA damaging chemotherapy and is associated with docetaxel resistance. Developed by Almac diagnostics using unsupervised clustering, this assay predicts loss of the fanconi anaemia pathway and detects the immune signal to the presence of abnormal DNA[5]. In addition it provides information on over 30,000 genes to support further biomarker development. Preliminary work has confirmed this methodology to be feasible in prostate core biopsies with a high success rate of 92% in obtaining high quality RNA suitable for analysis.

5.3.1 ANALYSIS OBJECTIVES

- To determine prevalence of DNA repair deficiency as determined by DDRD in men with newly diagnosed metastatic disease.
- To correlate with clinical outcome data in order to characterise the biomarker defined groups and determine the prognostic effect
- To investigate if DDRD status predicts docetaxel resistance

5.4 SELECTION CRITERIA

Samples will be identified from the STAMPEDE cohort selected according to the following criteria:

- Provided consent for the use of pre-treatment prostate tissue samples in additional research
- Available tissue suitable for biomarker analysis

In addition, three separate cohorts will be selected according to the following criteria for each analysis:

- Analysis 1 will include samples from men with non-metastatic disease randomised between control and docetaxel treatment
- Analysis 2 will include samples from men with newly diagnosed metasatic disease randomised to any treatment arm
- Analysis 3 will include samples from men with newly diagnosed metastatic disease randomised to control or docetaxel treatment and who joined the trial from November 2011 onwards when prostate core biopsies became mandatory

6 SAMPLE PROGRESSING

All samples will be identified from the STAMPEDE trial database and made available to this project via the trial biorepository, the Wales Cancer Bank. The samples will be allocated a study specific ID for the purposes of this project. Prior to transfer, the samples will be sectioned and reviewed by a pathologist in order to mark the dominant Gleason lesion.

For projects 1 and 3 the following samples will be required:

- Samples from 400 patients (200 docetaxel treated and 200 control). For each patient x4 5um slides will be provided with an additional H&E slide on which the dominant Gleason core is marked.

For project 2 the following samples will be required:

- Samples from 100 patients. For each patient 10 unstained slides will be provided (minimum 1x5mm).

Once analysis is complete any remaining tissue will be returned to the Wales Cancer Bank.

7 BIOMARKER ANALYSIS

7.1 PROSTATE PROGNOSTIC ASSAY

Archival tissue belonging to 400 patients (200 control and 200 docetaxel treated) will be analysed. For each sample the prostate core containing the dominant gleason lesion will be macrodissected for RNA extraction using the Roche High Pure RNA Parrafin Kit. Nanodrop spectrophotometric QC will be performed on all nucleic acids received or extracted to determine concentration and purity and the minimum RNA QC cut off criteria used will be 12.5 ng/µl. Microarray profiling will then be performed using Xcel® Array (418 arrays in total – 400 Prostate samples and 18 standard processing controls). The total amount of RNA required is 50ng (concentration 12.5 ng/µl). The cohort will be separated into molecular subtypes (high and low risk) according to pre-defined cut-offs based on preliminary work.

7.2 GENOMIC LOH

Archival tissue belonging to 100 patients from all treatment groups within the STAMPEDE trial will be analysed. DNA will be extracted and sequenced using Foundation Medicine next generation sequencing (NGS) approach, Foundation One®. This analyses a large panel of cancer-related genes including BRCA, ATM and other genes involved in DNA repair. Analysis of the sequence data will be performed to quantify the extent of loss of heterozygosity across the tumour genome and prostate cancer specific cut-offs for high and low genomic LOH defined. An feasibility assessment will be undertaken and the prevalance of high genomic LOH determined in men with treatment naïve de novo metastatic disease.

7.3 DDRD STATUS

Archival tissue belonging to 400 patients (200 control and 200 docetaxel treated) will be analysed. For each sample the prostate core containing the dominant gleason lesion will be macrodissected for RNA extraction using the Roche High Pure RNA Parrafin Kit. Nanodrop spectrophotometric QC will be performed on all nucleic acids received or extracted to determine concentration and purity and the minimum RNA QC cut off criteria used will be 12.5 ng/µl. Microarray profiling will then be performed using Xcel® Array (418 arrays in total – 400 Prostate samples and 18 standard processing controls). The total amount of RNA required is 50ng (concentration 12.5 ng/µl). DDRD status will be determine used pre-defined cut-offs based on preliminary work.

7.4 SUBSEQUENT ANALYSIS:

The results of all biomarker analysis will be transferred to the MRC CTU at UCL for correlation with clinical data. This will include characterising the biomarker defined subgroups according to current prognostic clinical features such as age, PSA, T-stage and Gleason grade. The prevalence of each biomarker will be determined and an assessment made as to the feasibility of each approach.

The differential clinical response will be determined for the cohorts tested with the prognostic and DDRD assays. This will use available FFS and OS data and will be visualised by Kaplan-Meier curves. The results will then be incorporated to predict associations with patient response using univariate and multivariate analysis independent of the known prognostic clinical factors.

8 STATISTICAL CONSIDERATIONS

A formal sample size calculation to determine the power to detect a prognostic and predictive effect is not possible without knowing the prevalence of each biomarker in this population. This is a key aim of the project. The following scenarios illustrate the power this exploratory study will have based on an *estimated* biomarker prevalence of 15-25%.

8.1 INVESTIGATING THE PROGNOSTIC EFFECT OF BIOMARKER STATUS

The following assumptions have been made:

- FFS data known for 70% in Q2 2016
- Median FFS in M1 patients in control arm is 12 months

Table 1 Minimal detectable difference based on biomarker prevalence of 15%

NO OF CONTROL SAMPLES	REQUIRED EVENTS IN BM-VE POPULATION (ASSUMED TO BE N~150)	POWER	ALPHA	MINIMAL DETECTABLE DIFFERENCE (HAZARD RATIO)
200	107	90%	0.05	1.7
200	118	85%	0.05	1.6
200	118	80%	0.05	1.55

A Hazard Ration of 1.7 equates to a 5 month difference in FFS i.e. the median FFS in the biomarker postiive population of 7 month vs. the biomarker negative population (control) of 12 months.

Table 2 Minimal detectable difference based on biomarker prevalence of 25%

NO OF CONTROL SAMPLES	REQUIRED EVENTS IN BM-VE POPULATION (ASSUMED TO BE N~170)	POWER	ALPHA	MINIMAL DETECTABLE DIFFERENCE (HAZARD RATIO)
200	122	80%	0.05	1.75
200	121	75%	0.05	1.7

8.2 INVESTIGATING THE PREDICTIVE EFFECT OF BIOMARKER STATUS

To determine the predictive effect of biomarker status, the effect of treatment is compared between biomarker positive patients in the docetaxel and control groups. The hypothesis is that marker positive patients may benefit less from docetaxel treatment (i.e. negative predictive biomarker) and therefore the analysis needs to be powered to detect a smaller clinical difference in a sub-set of patients, estimated to be between 15 and 25% of all those sampled.

The following assumptions have been made:

- FFS data known for 70% in Q2 2016
- Median FFS in M1 patients in control arm is 12 months

Table 3 Minimal detectable prognostic effect

NO OF SAMPLES	REQUIRED CONTROL EVENTS	POWER	ALPHA	MINIMAL DETECTABLE DIFFERENCE
400	153	90%	0.15	0.76
400	147	85%	0.15	0.78
400	149	80%	0.15	0.8

The STAMPEDE data demonstrated a median FFS benefit of docetaxel in the M1 sub-group of 7 months, equivalent to a 38% improvement (Hazard Ratio = 0.62). If, as hypothesised, biomarker positive patients benefit less from docetaxel the effect size will be smaller. Based on the assumptions specified above, sampling 400 patients provides 80% power to detect a 20% improvement; equivalent to 2.4 months, which has been selected as the minimal clinically significant clinical difference we seek to determine.

9 FUTURE PROGRAMME OF WORK

The feasibility and prevalence data generated by this sub-study will be shared with the STAMPEDE TMG. It is highly relevant to the ongoing trial development aiming to incorporate biomarker analysis within the STAMPEDE trial platform, enabling novel targeted therapeutic strategies to be evaluated in biomarker defined sub-groups. In additional it is hoped that through identifying poor prognostic groups, alternative treatment strategies can be evaluated in those with the highest unmet need.

10 REFERENCES:

1. James, N.D., et al., *Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial.* Lancet, 2015.
2. *NHS England - Clinical Commissing Policy Statement: Docetaxel in combination with ADT for treatment of hormone naive metastatic prostate cancer* 2016.
3. Watkins, J.A., et al., *Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers.* Breast Cancer Res, 2014. **16**(3): p. 211.
4. Abkevich, V., et al., *Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer.* Br J Cancer, 2012. **107**(10): p. 1776-82.
5. Mulligan, J.M., et al., *Identification and validation of an anthracycline/cyclophosphamide-based chemotherapy response assay in breast cancer.* J Natl Cancer Inst, 2014. **106**(1): p. djt335.

2016.06.28 re-issue: removal of reference to HRA Approval

Health Research Authority

West Midlands - Edgbaston Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

22 April 2016

Dr Clare Gilson
Clinical Research Fellow
University College London
MRC CTU at UCL
Aviation House, 125 Kingsway
London
WC2B 6NH

Dear Dr Gilson

Study title:	**Developing prognostic and predictive biomarkers in prostate cancer using archival tissue from STAMPEDE participants: a sub-study of STAMPEDE**
REC reference:	**16/WM/0188**
IRAS project ID:	**203620**

The Proportionate Review Sub-committee of the West Midlands - Edgbaston Research Ethics Committee reviewed the above application on 20 April 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Adam Garretty, NRESCommittee.WestMidlands-Edgbaston@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

NHS
Health Research Authority

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Summary of discussion at the meeting

Social or scientific value; scientific design and conduct of the study
The sub-committee noted the researchers had sought advice from the Chair of the Edgbaston Committee before submitting the application. The sub-committee noted they are familiar with the STAMPEDE trial which is a good study with supporting information always laid out well.

Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)
The sub-committee noted this is a minimal risk study.

Informed consent process and the adequacy and completeness of participant information
The sub-committee discussed the consent procedure and agreed with the researchers that it is not appropriate to go back to participants who may be in a poor state to gain consent to use samples as this would cause more distress.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Covering letter on headed paper [Cover letter]		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [STAMPEDE UCL Insurance certificate]		
IRAS Checklist XML [Checklist_30032016]		30 March 2016
IRAS Checklist XML [Checklist_05042016]		05 April 2016
Participant information sheet (PIS) [STAMPEDE PIS and ICF by version]	1.0	
REC Application Form [REC_Form_30032016]		30 March 2016
Research protocol or project proposal [Biomarker development in prostate cancer: a sub-study of STAMPEDE Protocol]	1.0	18 March 2016
Summary CV for Chief Investigator (CI) [CV (Clare Gilson, CI)]		
Summary CV for supervisor (student research) [CV (Matt Sydes, supervisor)]		

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research

Health Research Authority

Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

16/WM/0188	**Please quote this number on all correspondence**

Yours sincerely

pp Adam Connell

Mr Paul Hamilton
Chair

Email: NRESCommittee.WestMidlands-Edgbaston@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Health Research Authority

"After ethical review – guidance for researchers" [SL-AR2]

Copy to: *Professor Max Parmar*

Health Research Authority

West Midlands - Edgbaston Research Ethics Committee

Attendance at PRS Sub-Committee of the REC meeting on 20 April 2016

Committee Members:

Name	*Profession*	*Present*
Mr Chris Foy	Medical Statistician	Yes
Mr Paul Hamilton	Retired Local Government Officer	Yes
Professor John Marriott	Pharmaceutical Chemist/Academic Pharmacist	Yes

Also in attendance:

Name	*Position (or reason for attending)*
Mr Adam Garretty	REC Assistant
Helen Poole	REC Manager

FoundationOne SPECIMEN GUIDELINES

FOUNDATIONONE®

FoundationOne can help you determine next steps for the care of your patients by accurately detecting all classes of genomic alterations. Below are Specimen Guidelines to help ensure successful genomic profiling.

Selecting the Best Specimen from Multiple Options

Has the patient been treated with targeted therapy?

NO →
Use recurrence or original resection (most recent material preferred).
Metastasis biopsy or primary tumor acceptable (choose site with highest percent tumor or largest tumor focus).

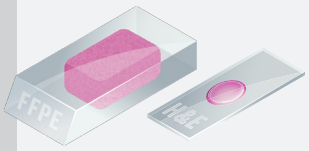
YES →
MUST use post-targeted therapy specimen, if available.

Acceptable Samples

- FFPE specimens, including core needle biopsies, fine-needle aspirates and effusion cytologies.
- Tissue should be formalin-fixed, paraffin embedded. Use standard fixation methods to preserve nucleic acid integrity. 10% neutral-buffered formalin for 6-72 hours is industry standard. DO NOT use other fixatives (Bouins, B5, AZF, Holland's).
- **Do not decal.** When decalcification is required, EDTA is recommended. Do not use strong acids (e.g. hydrochloric acid, sulfuric acid, picric acid).

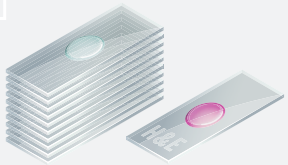
1 SAMPLE TYPES

When feasible, please send the block + 1 original (not recut) H&E slide.



OR

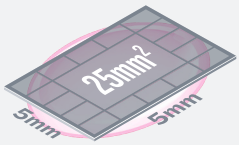
10 unstained slides (positively charged and unbaked at 4-5 microns thick) + 1 original (not recut) H&E Slide.



2 SAMPLE SIZE SURFACE AREA

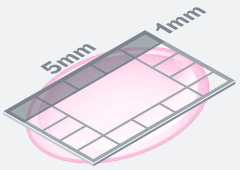
Optimal: 25 mm²

If sending slides, provide 10 unstained slides cut at 4-5 microns thick.



Minimal: 5 mm²

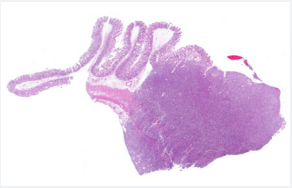
For small (<25mm²) or impure samples, additional unstained slides may be needed to extract sufficient DNA for testing.



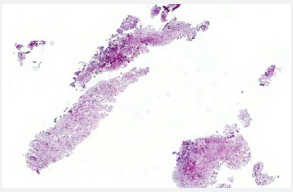
3 TUMOR NUCLEI PERCENTAGE

Optimal: 30% Minimal: 20%

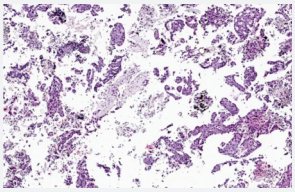
Percent tumor nuclei = number of tumor cells divided by total number of all cells with nuclei.*



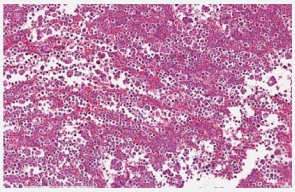
Resection



Small Biopsy



Fine Needle Aspiration (Cell Block)



Fluid Exfoliative Cytology (Cell block)

* Liver specimens may require additional tumor.

SHIPPING INSTRUCTIONS

1. Place the samples, FoundationOne Requisition Form, pathology report, insurance information, and any other attachments into the FoundationOne Specimen Kit.
2. Place the specimen kit (including samples and paperwork) into the provided shipping pack and seal the shipping pack.
3. Complete the pre-printed shipping labels (if necessary) and apply to shipping pack.
4. Ship sealed shipping pack to:

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   150 Second Street
   Cambridge, MA 02141

Drop the package at your site's designated FedEx pick up location or call +1.800.309.0530 to request a pick up.

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Overview

Incorporating Biomarker Stratification into STAMPEDE: an Adaptive Multi-arm, Multi-stage Trial Platform

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Abstract

The treatment and outcomes for advanced prostate cancer have experienced significant progress over recent years. Importantly, the additional benefits of 'up front' chemotherapy (docetaxel) and abiraterone, over and above conventional androgen deprivation, have been separately demonstrated in the multi-arm, multi-stage (MAMS) STAMPEDE protocol, which continues recruitment to other questions. Alongside this, insights into the underlying molecular biology and, inevitably, the molecular heterogeneity of prostate cancer are opening the door to new therapeutic approaches. Incorporating this understanding and testing these hypotheses within STAMPEDE brings new challenges to the MAMS approach, but has the potential to further improve the outlook for this disease.

Key words: Biomarkers; clinical trial design; platform trials; precision medicine; prostate cancer

Statement of Search Strategies Used and Sources of Information

This overview reflects the opinion and experience of the authors and evidence has been presented accordingly. It is based upon our own research findings and clinical trial experience. It is not a systematic review.

Introduction

The last decade has seen major advances in both the understanding and the treatment of advanced prostate cancer, with a number of agents gaining approval as standard of care (SOC) in castrate-resistant disease. More recently, the additional benefit of using these drugs earlier, at first presentation, has also been shown and is now considered SOC [1–4]. In parallel, there has been significant progress in understanding prostate cancer biology that promises to further improve outcomes, but by nature moves away from a 'one size fits all' approach. Implementing precision medicine requires the validation of putative predictive biomarkers within well-designed clinical trials. Analysis of representative samples obtained as part of a clinical trial protocol can support many stages of biomarker discovery, assay development and qualification, as laid out in the Cancer Research UK Biomarker Roadmap [5]. Knowledge gained through the genomic characterisation of advanced metastatic prostate cancer provides both the rationale and the means to progress this research priority in the first-line setting.

It has been shown that around 20% of metastatic castrate-resistant prostate cancers (mCRPC) have loss-of-function somatic genomic aberrations or germline deficiencies in genes involved in DNA repair, in particular those involved in the repair of double-stranded DNA breaks using homologous recombination. The resulting homologous recombination deficiency (HRD) supports synthetic lethality as a therapeutic approach in prostate cancer. Importantly, the poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib, has been shown to benefit this group,

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providing the proof-of-concept for this precision approach [6,7].

However, the greatest absolute benefit of effective therapies may be observed when used early, at the initiation of long-term androgen-deprivation therapy (ADT) [1–4]. Furthermore, prostate cancers exhibit significant intratumoural genetic heterogeneity, which increases in advanced disease in response to multiple lines of therapy [8,9]. Mechanistically, DNA repair-deficient cancers can be expected to acquire and tolerate somatic mutations at a greater rate, which may thwart targeted precision medicine approaches due to the increasing likelihood of acquired secondary resistance and risk of sampling bias due to the spatial genetic heterogeneity observed in metastatic prostate cancer [10,11]. Together, this provides the rationale to evaluate precision medicine approaches earlier, with the aim of achieving the greatest impact on patient outcome.

Approval from Cancer Research UK and independent scientific peer-review has been obtained to evaluate rucaparib within the STAMPEDE trial platform. Here we set out the considerations and challenges faced when incorporating biomarker stratification within an adaptive trial platform, which will be the first example of a biomarker-directed treatment strategy in this disease setting.

STAMPEDE

STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy) is a well-established randomised controlled trial that recruits men with high-risk locally advanced or metastatic prostate cancer who are commencing long-term ADT for the first time, termed hormone-naive prostate cancer (HNPC) [12]. The trial uses a multi-arm multi-stage (MAMS) platform design: multi-arm because many treatment approaches can be tested simultaneously; multi-stage because pre-specified interim analyses can be used to stop recruitment early to arms showing insufficient evidence of activity [13]. Data from all stages are included in the final analysis of efficacy, powered on the primary outcome, overall survival. The trial opened in 2005 with five 'original comparisons' evaluating the efficacy of adding docetaxel, zoledronic acid and celecoxib, given alone or in combination, to the then SOC, ADT ± prostate radiotherapy. Since then a number of new research arms have been added to undertake randomised comparisons of: abiraterone; prostate radiotherapy for patients with newly diagnosed metastatic disease (M1|RT); enzalutamide given in combination with abiraterone; metformin, an anti-diabetic medication; and, most recently, transdermal oestradiol, a proposed alternative form of ADT (see Figure 1).

Comparisons: Reported, Unreported and Ongoing

The results of the 'original comparisons' and the 'abiraterone comparison' have been reported, with both docetaxel and abiraterone shown to significantly improve survival [2,3]. The addition of docetaxel improved median survival from 71 months to 81 months; hazard ratio 0.78 (95% confidence interval 0.66–0.93); $P = 0.006$. The strength of evidence is most clearly apparent within the metastatic subgroup where the benefit is also reflected in the results of the CHAARTED trial. Both trials contributed to

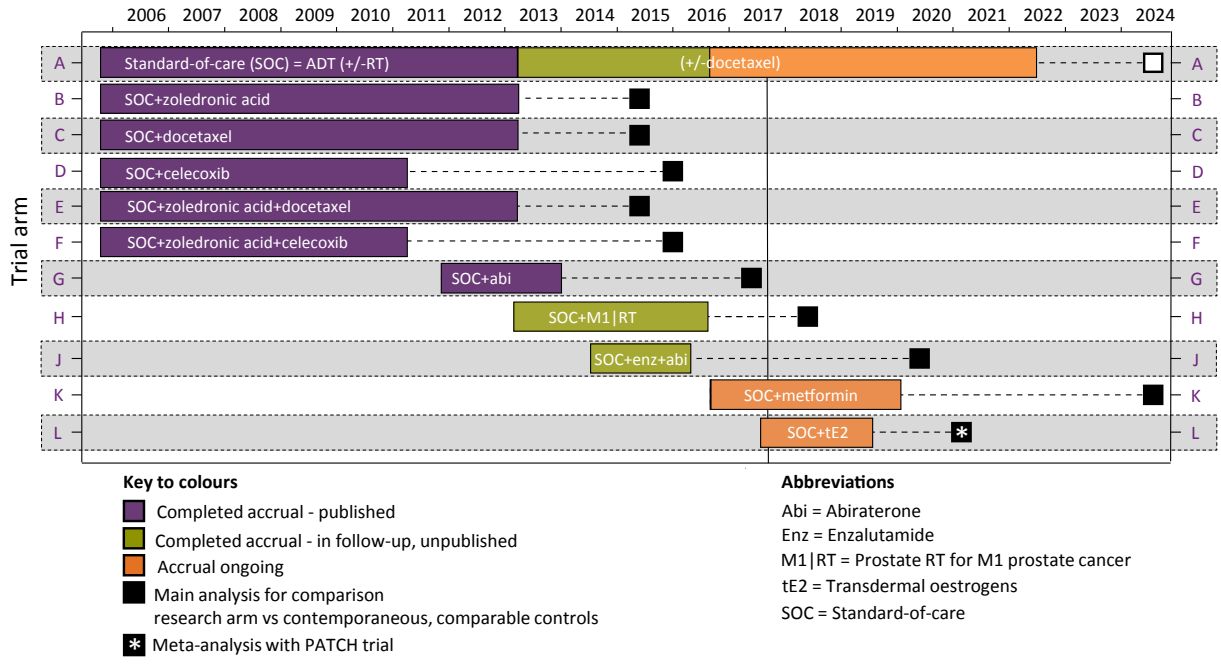

Fig 1. Arms of the STAMPEDE trial platform over time.

the STOpCaP meta-analysis; aggregate data from 3206 patients with metastatic HNPC showed that docetaxel improved 4 year survival by 9% (hazard ratio 0.77; 95% confidence interval 0.68–0.87; $P < 0.0001$) [14]. These data have been practice changing [15]. The results of the 'abiraterone comparison' also show improved outcome; 3 year survival improved from 76% to 83%; hazard ratio 0.63 (95% confidence interval 0.52–0.76) [3]. Opportunistic data acquired through the overlapping randomised population accrued between 2011 and 2014 were presented at ESMO 2017 and suggest superior progression-free survival with abiraterone but a comparable survival outcome [16]. The 'M1|RT comparison' and 'enzalutamide and abiraterone comparison' continue in follow-up, with survival results expected in the next 1–3 years. Recruitment is ongoing to two comparisons evaluating metformin and transdermal oestradiol as re-purposed anti-cancer therapies, both proposed to mitigate the adverse cardiovascular and metabolic effects of long-term androgen suppression [17,18]. STAMPEDE is investigating whether adding metformin to the current SOC for non-diabetic men can improve all-cause survival, and whether transdermal oestradiol, shown to offer superior cardiovascular, quality-of-life and bone health outcome, is non-inferior based on survival [19–21].

Rationale to Incorporate Biomarker Selection within STAMPEDE

The 'rucaparib comparison' will be embedded into STAMPEDE by way of a protocol amendment as the most efficient route to address this question in this disease setting (see Figure 2). Through further adaptation, we avoid establishing a competing biomarker-selected trial within an overlapping population, which could risk impacting on accrual and potentially generalisability of results through depletion of biomarker-defined groups hypothesised to have both prognostic and predictive effects. Through incorporation we can determine and control the impact on the ongoing comparisons and continue to answer important research questions for both biomarker-positive and -negative patients through an inclusive trial platform. This is key to sustaining efficient accrual to all comparisons and conducting cost-effective evaluation of new agents, particularly for those targeted at low-frequency biomarkers.

Biomarker Stratification: Considerations

When planning biomarker-enriched clinical trials requiring prospective biomarker characterisation there are several aspects that require consideration to inform the trial design and feasibility of implementation (summarised in Table 1).

Biomarker Measurement

The reliable identification of the biomarker-positive population of interest requires an analytically validated assay shown to perform to an acceptable standard in clinically available samples [22]. In the case of the STAMPEDE trial population, these are typically small, prostate core biopsies stored as formalin-fixed, paraffin-embedded tumour blocks. Preliminary data have highlighted the

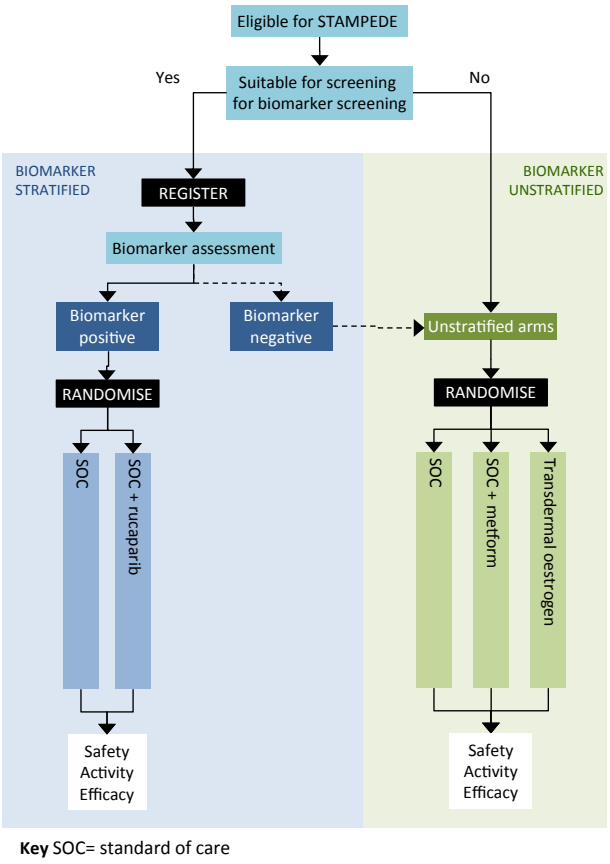

Fig 2. Future STAMPEDE randomisation schema.

Table 1
Considerations for biomarker-enriched trial designs

Framework for incorporating biomarker stratification in a platform trial
1. Can the biomarker of interest be reliably measured using a validated assay?
2. What is test-performance in clinically available samples representative of the population of interest?
3. Is the biomarker prognostic necessitating a separate control in order to distinguish a prognostic from a predictive effect?
4. What is the biomarker prevalence in the population of interest?
5. What is the strength of evidence of a predictive effect, i.e. the specificity of the biomarker?
6. What is the strength of evidence to support the rationale and clinical efficacy of the targeted therapy in the biomarker-defined group?
7. What is the overlap between this biomarker-defined group and others of interest?
8. What are the implications for other overlapping accruing comparisons?

impact of pre-analytical variables such as variation between sites' fixation protocols in the ability to extract sufficiently high-quality DNA required for analysis. This has been recognised by others; Genomic England have reported that the quality of routinely obtained cancer samples may often be suboptimal for molecular analysis and are leading several initiatives aiming to improve sample quality and inform the most suitable methods for sample collection and storage [23]. The clinical utility of the method of biomarker measurement should be assessed in representative clinical samples to estimate the test-failure rate. This in turn informs the numbers needed to screen, a crucial parameter in defining the cost, accrual time and, therefore, feasibility of implementation. The impact of a high test failure rate is greatest when aiming to evaluate low-prevalence biomarkers when this can render a trial infeasible.

To be workable within a clinical trial screening window the biomarker assessment must be available within a reasonable timeframe. Several factors will affect turnaround around times, including pathology department resources, shipping distances, the capacity of the biomarker provider and the need to batch for cost-efficiency. The risk of a long turnaround time is that randomisation and, therefore, treatment, is delayed. The effect of this will probably be greatest in trials recruiting patients with progressive disease where a participant's clinical status may change, making them ineligible during the time taken to complete screening. However, in all disease settings, the time required to undertake biomarker analysis should not risk disadvantaging patients' access to treatment. In trial platforms where multiple randomisations are possible, biomarker analysis should be completed in such time that patients remain eligible for all possible randomisations. This is relevant for STAMPEDE, which will continue to randomise to non-biomarker selected comparisons.

Prognostic Importance

Aberrations in BRCA and other HRD genes are negatively prognostic in prostate cancer and, therefore, patients allocated to the research arm of the 'rucaparib comparison' will only be evaluated against comparable biomarker-positive controls [24–27]. Evidence of a prognostic effect can inform the design of biomarker-enriched trials and affect the assessment of feasibility, particularly of low-frequency biomarkers. When considering a biomarker-selected randomisation within a platform trial, knowledge of a prognostic effect informs whether it is justifiable to share a control arm, with resulting efficiencies (namely fewer control arm patients). If the biomarker is known to be prognostic, separate comparisons are required in order to distinguish a predictive from a prognostic effect, as exemplified by the design of FOCUS-4 [28]. Knowledge of a prognostic effect can also inform the size of a trial powered to detect a difference in time-to-event outcomes. If the survival time is shorter, the information required for reliable analyses (e.g. number of deaths for a trial powered on the primary outcome of survival) will be accrued sooner and, therefore, a smaller trial may be required.

However, the acquisition of robust prognostic information for emerging biomarker-defined groups can be challenging. Retrospective retrieval of archival samples is vulnerable to bias, with the risk that those patients who have subsequently progressed and enrolled on mCPRC trials requiring archival tissue analysis are under-represented. Additionally, it has been suggested that the quality of the DNA extracted from formalin-fixed paraffin-embedded samples declines with sample age; this has been supported by the experience of the STAMPEDE group to date. It is hoped that through data sharing, for example through contribution to genetic consortia, it will be possible to inform clinical trial design and target research efforts in poor prognostic genetic subpopulations, aiming to improve outcome in those patients with the greatest unmet clinical need.

Biomarker Prevalence

The duration of recruitment to the 'rucaparib comparison' will depend on the frequency of DNA repair defects in men eligible for STAMPEDE. Evidence from mCRPC cohorts and primary localised prostate cancer has that shown DNA repair defects are more common in metastatic disease [8,29–32]. Around 20% of mCRPC cohorts (range 7–27%) have detectable mutations in one or more of 14 genes involved in homologous recombination, including BRCA1, BRCA2, ATM, BARD1, CHEK2, PALB2, RAD51 [6,7,9,33–40]. The vast majority of the prostate cancer cohorts profiled to date have either consisted of men with advanced, heavily pre-treated CRPC or localised prostate cancer suitable for radical prostatectomy. Therefore, knowledge of the genomic landscape of men presenting with high-risk locally advanced or metastatic prostate cancer (i.e. eligible for STAMPEDE) is currently very limited. Furthermore, all sequenced mCRPC series to date involve patients participating in trials, precision medicine initiatives or autopsy programmes at tertiary academic centres and are, therefore, vulnerable to selection bias (see Table 2) [6,7,9,34,36,37].

Eligibility to the 'rucaparib comparison' will be limited to metastatic HNPC with a detectable pathogenic mutation in one or more of 14 HRD-related genes and the trial design has been developed based on an estimated biomarker prevalence of 10–15%. A feasibility assessment is planned 1 year after the activation of recruitment; accumulating prevalence data acquired in the screened population will be reviewed and adjustments to the target screening accrual numbers made accordingly.

The frequency of the biomarker of interest in the target population is crucial in developing the best approach to therapeutic evaluation within a biomarker-enriched trial. Trials restricting enrolment to low-prevalent biomarker groups risk being very costly, given the numbers needed to screen; additionally, the high screen failure rates can deter patients and investigators, negatively impacting on accrual. This contributes to the rationale for incorporating both biomarker-selected and -unselected randomisations within

Table 2
DNA repair deficiency in prostate cancer: summary of prevalence data

Ref	Cohort details	M0 (n)	M1 (n)	% BRCAm	% HRD*
[30]	HNPC suitable for prostatectomy	112	-	1%	4%
[31]	Low/intermediate risk HNPC	333	-	4%	13%
[29]	Mixed HNPC	55	2	0%	11%
[32]	Mixed cohort, predominantly M0	181	37	0	0
[8]	Mixed cohort, both HNPC and mCRPC	25	20	12%	20%
[29]	Mixed, fatal mCRPC sampled at rapid autopsy and HNPC suitable for prostatectomy	11	50	2%	7%
[34]	Selected due to unusual clinical course, suspected predisposition, e.g. family history or atypical histology	29	13	16% (10% gBRCA)	27% (24% gHRD)
[36]	Fatal mCPRC sampled at rapid autopsy		54	7%	16%
[6]	mCRPC trial participants at academic centres	-	150	14%	23%
[7]	mCRPC in an unselected PARPi trial	-	50	14%	27%
[37]	Cohorts participating in clinical trials, rapid autopsy programmes or precision medicine initiatives at academic centres		692	6.2% (gBRCA)	11.2% (gHRD)
[35]	Sporadic mCRPC eligible for abiraterone +/- PARPi	-	80		25%
[33]	Sporadic mCRPC eligible for PROREPAIR-B (prospective cohort study)	-	419	4.2% (gBRCA)	9.1% (gHRD)

BRCAm = BRCA mutant, CNA = copy number alteration, gBRCA = germline BRCA mutation, gHRD = germline HRD mutation, HNPC = hormone-naïve prostate cancer, M0 = non-metastatic prostate cancer, M1 = metastatic prostate cancer, tNGS = targeted next generation sequencing, mCRPC = metastatic castrate resistant prostate cancer, PARPi = PARP inhibitor, WES = whole exome sequencing.
*Homologous recombination deficiency defined as pathogenic aberration in one or more: BRCA1, BRCA2, ATM, BARD1, BRIP, CDK12, CHEK2, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD54L.

one trial platform, in order to sustain accrual to low-prevalence biomarker groups.

Evidence of a Predictive Effect

Eligibility for the 'rucaparib comparison' will be restricted to the biomarker-positive population based on the evidence of a predictive effect demonstrated for PARP inhibition in mCRPC. Justification of biomarker enrichment requires evaluating the strength of evidence of a predictive effect; this also informs the effect size [41]. In the scenario that the evidence of a predictive effect is judged insufficient to limit treatment to only biomarker-positive patients, it may be preferable to enrol an unselected population and, through prospective biomarker assessment, stratify by biomarker status to enable pre-planned subgroup analyses. If, however, there is good evidence of a predictive effect, such that enrichment can be justified, randomisation may be limited to the biomarker population. One approach to the challenge of defining the level of evidence required is to adopt a pragmatic approach: would randomisation of an unselected population be acceptable and, therefore, feasible? Here, evaluation within an adaptive MAMS trial platform is advantageous as it is possible to initially randomise the population with the strongest evidence of a predictive effect and then subsequently activate randomisation in a broader group should sufficient activity be shown. Early stopping rules could be used in this scenario, but the value of being able to test the specificity of the marker is high: it may be that the research treatment could offer a broader benefit, which risks being missed if an enrichment design is adopted and not subsequently evaluated.

The magnitude of the targeted treatment effect is also dependent on the evidence of a predictive effect; where there is strong evidence of a predictive effect in a biomarker-defined group, the treatment effect may be expected to be greater than for a non-targeted therapy in an unselected population. The vast majority of data demonstrating the efficacy of PARP inhibition have been acquired in the setting of ovarian or breast cancer and the strongest evidence of a predictive effect has been shown for inactivating mutations in BRCA1 and BRCA2 [42–45]. Preliminary evidence of an anti-tumour effect has been shown for mCRPC, with defects in several homologous recombination genes, including BRCA2, ATM and CHEK2 [7]. However, as ATM and CHEK2 mutations have not been described in other BRCA-associated cancers to date, here the evidence of a predictive effect is judged to be less, limited to prostate cancer and pre-clinical data [7,40]. A balance is required in order to evaluate the treatment in the broadest patient group hypothesised to benefit, while accepting that where there is less evidence of a predictive effect, it may be appropriate to target a smaller treatment effect, thus requiring a larger randomised population. It should be considered whether to include a pre-planned subgroup analysis in the group(s) with the strongest evidence of a predictive effect; a step-down approach to analysis may be taken, as shown by other PARP trials [44,46].

Therapeutic Relevance

Only through pairing the biomarker-defined population with an effective therapeutic strategy can precision medicine approaches improve outcome. However, biomarker-treatment pairings may fail to translate to patient benefit due to an incomplete understanding of the biology of the biomarker or the interaction between the biomarker and the therapy [47]. Metastatic prostate cancer has been shown to be associated with multiple genetic aberrations, but in order to realise the therapeutic potential of this, it is necessary to distinguish mutations of significance. Ultimately, the highest level of evidence supporting an identified genetic alteration as an oncogenic driver and, therefore, a valuable therapeutic target, requires prospective evaluation within a clinical trial, including demonstration that biomarker-negative cohorts do not benefit from the targeted therapy [48]. Such evidence needs to be tumour site-specific due to the concept of epistasis, the gene–gene interactions proposed to explain the observed attenuated biological consequences of specific genetic aberrations according to tumour type. Examples of this include the differing impact of a BRAF V600E mutation, predictive of sensitivity to vemurafenib in melanoma and dabrafenib in non-small cell lung cancer, but not in the context of colorectal cancer. The latter is proposed to be due to the feedback of epidermal growth factor receptor, thus emphasising that driver classification requires contextual knowledge of other mutations present [49,50].

One of the most well-described and seemingly perplexing challenges to the implementation of precision medicine approaches is intratumoural heterogeneity [51]. This can be considered as either spatial, the variation between different sites of disease, or temporal, the variation at different time points, for example pre- and post-treatment [52]. The key challenge to implementing precision medicine in metastatic prostate cancer is spatial heterogeneity, as this has the potential to introduce sampling bias [51]. Evidence from multi-regional sampling of cases of mCRPC obtained at rapid autopsy and sequential sampling shows metastatic spread to be polyclonal and polyphyletic, with evidence of metastatic-to-metastatic spread and both spatial and temporal heterogeneity [10]. This provides a powerful rationale to evaluate precision approaches in the first-line treatment of metastatic HNPC at the point closest to the sampling of the primary tumour. However, evidence of spatial heterogeneity will continue to motivate the investigation of alternative approaches to biomarker assessment, such as circulating tumour DNA. Such approaches are particularly relevant to this disease setting, given the predominance of bone metastatic involvement, which remains challenging to sample adequately to allow genetic analyses [53].

Overlapping Biomarkers of Interest

In order for a trial platform such as STAMPEDE to evaluate multiple biomarker–therapeutic pairings, knowledge of the overlap between predictive biomarkers is required. This requires systematic profiling of a representative population; ongoing work being conducted as part of STAMPEDE aims to inform this. Genomic characterisation of clinical trial cohorts has been invaluable in other disease settings, such as those conducted as part of the S-CORT programme associated with FOCUS-4, which have informed biomarker prevalence, prognostic impact and overlap [54,55]. If, for example, all cases of HRD prostate cancers overlap with a second biomarker of interest, then the feasibility of accruing to both comparisons would be dependent on the prevalence of both biomarkers or expanding accrual internationally. Greater understanding of the genetic profile of high-risk or metastatic prostate cancer will be crucial in identifying and designing future potential comparisons to be assessed in STAMPEDE.

What Next for STAMPEDE?

In preparation for activating randomisation to the 'rucaparib comparison', a biomarker-screening pilot will start in late 2017. This will aim to establish the necessary processes to obtain rapid, prospective sequencing data prior to randomisation in order to determine eligibility. Following the pilot phase, biomarker screening will be activated in all participating centres when randomisation opens in early 2018. The development of this, the first biomarker-selected comparison, has highlighted the requirement for preliminary biomarker-focused research to inform the implementation of such approaches. The STAMPEDE protocol has included participant consent for the collection and analysis of archived tumour samples. As more outcome data become available there are a growing number of opportunities to support biomarker development through correlative analysis. Funded by Prostate Cancer UK, the STRATOSPHere consortium (STratification for RAtional Treatment-Oncomarker pairings of STAMPEDE Patients starting long-term Hormone treatment) aims to undertake a co-ordinated, multi-centre approach to generating preliminary data to accelerate the introduction of biomarker-selected comparisons with STAMPEDE.

One such future comparison in development aims to evaluate the addition of a checkpoint inhibitor in men with metastatic HNPC. Recognising that there are currently insufficient data to support the use of a predictive biomarker-enrichment strategy, an alternative approach to biomarker development is suggested. As part of the STRATOSPHere consortium, a parallel translational programme would aim to prospectively collect and characterise the randomised population. Then, if supported by external data, prospective enrichment may subsequently be implemented as part of the multi-stage design. Alternatively, it may be possible to power a subgroup analysis by biomarker status, based on the prevalence data acquired. Finally, in order to accelerate biomarker validation it will be important to establish the necessary infrastructure that allows clinical and molecular characterisation and endorses data sharing.

Conclusion

The management of men with high-risk prostate cancer has lagged behind other tumour types where molecular characteristics routinely inform therapeutic choice. Knowledge gained from the genomic characterisation of CRPC cohorts, together with the evidence of the therapeutic relevance of DNA repair defects, provides the rationale to investigate a precision approach to treatment. Through further adaptation, STAMPEDE will evaluate the addition of a PARP inhibitor in this, the disease setting in which the greatest impact of outcome has been shown to date. The implementation of precision medicine approaches in low-frequency biomarker groups is challenging, but incorporating both biomarker-selected and -unselected randomisations within a single platform offers further efficiencies to the MAMS approach and ensures that the trial platform remains inclusive and attractive to patients, investigators and funders. Correlative translational analyses using STAMPEDE data offer unparalleled opportunity for biomarker development, which can continue to inform the trial design and generate the required preliminary data as laid out in the framework presented in this review. Through this latest adaption, we aim to ensure STAMPEDE remains innovative and continues to accelerate the acquisition of knowledge that will improve outcomes for men affected by high-risk prostate cancer.

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